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7148 C*

Determination of Plasma Bilirubin; a Modified van den Bergh Procedure.

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The diazotization of bilirubin in the van den Bergh estimation is strictly proportioned to the intensity of the color produced.[1] However, the use of the artificial color standards and the stratification of the plasma-ammonium sulphate and alcohol in the determination afford a valid criticism of the procedures ordinarily employed. Some three years ago we adapted the Thannhauser-Anderson[2] method to avoid these difficulties. A strongly acidified solution of azobilirubin, which remains unchanged for 3 to 6 months if kept in a cold refrigerator, is employed as a standard. A proportional concentration of acid is added to the unknown. There is no stratification of the mixture, and the red reaction changes to an intense blue.[3] Publication of our procedure has been delayed until a ready source of bilirubin for the standard could be located. Failing in this, we have prepared our own bilirubin from bile rather simply. Foweather[4] recently has given a short procedure for obtaining bilirubin from gall stones. Our method of preparing bilirubin and the technique for the quantitative test follow:

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* P represents a preliminary, C a complete manuscript.

[1] Hunter, G., *Brit. J. Exp. Path.*, 1930, **11**, 407.

[2] Thannhauser, J. A., and Anderson, E., *Deutsch. Arch. f. klin. Med.*, 1921, **137**, 179.

[3] Newman, C. E., *Brit. J. Exp. Path.*, 1928, **9**, 112.

Preparation of bilirubin. 500 cc. of bile from surgical drainage patients or 50 cc. of post mortem gall bladder bile (diluted 2-3 times) is allowed to stand a few hours in the refrigerator and the supernatant fluid is decanted. This is diluted several times with water. Barium chloride solution is added with stirring. If the precipitate of barium bilirubinate does not flocculate immediately, addition of a few drops of alkali usually suffices. When the precipitate settles the supernatant fluid is siphoned off. The precipitate is poured on a filter and is washed with water on the paper; it is air dried and pulverized in a mortar. The powder is extracted with warm alcohol and with ether or chloroform and again air dried. The precipitate is moistened with 10% sulphuric acid solution. It is then washed 3 times by suspension in a little absolute alcohol; we do this in 50 cc. centrifuge tubes, centrifuging and pouring off the alcohol. The residue is twice treated in a flask with boiling chloroform, filtered, and the chloroform evaporated with avoidance of over-heating towards the end. The bilirubin so obtained is rubbed into glacial acetic acid, centrifuged, the acid drained off, and the procedure repeated; the residue is air dried. It is again re-dissolved in boiling chloroform, filtered and evaporated to dryness. The final product (40 to 100 mg.) is brick red in color, is free from ash, and is quite stable. Standard solutions of bilirubin so prepared have checked with one another and with a supposedly pure German preparation (Schuchardt).

Preparation of the bilirubin standard. Ten mg. of bilirubin are dissolved in a little water with the aid of a few drops of 10% NaOH solution and the volume made up to 100 cc. To this is added 50 cc. of freshly prepared Ehrlich's diazo reagent, 100 cc, of saturated ammonium sulphate solution, and 400 cc. of alcohol. When the red color has developed, 100 cc. of concentrated HCl are poured in. The standard should be allowed to stand a few hours before using. It should be kept in the refrigerator and replaced after 3 months.

Determination of plasma bilirubin. To 2 cc. of plasma or serum, add 1 cc. of freshly mixed Ehrlich's reagent and mix. Note whether a "direct reaction" is obtained. Then add 2 cc. of saturated ammonium sulphate solution, 8 cc. of alcohol, and mix. When the red color has developed, add 2 cc. of concentrated HCl, mix, and let stand for some minutes. Then filter through a pleated paper. The filtrate is placed in the colorimeter and the cup set at 10 mm. The standard is racked to match the unknown, the reading in mm. is the mg. of bilirubin per 100 cc. of the plasma.

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[4] Foweather, F. S., *Biochem. J.*, 1932, **26**, 165.

Some 24 comparative determinations by our procedure and by the Thannhauser-Anderson method on the same specimen, using a cobalt standard and figuring the result as mg. of bilirubin, have given results ranging from 0.8 mg. to 30 mg. for the new method against 0.4 mg. to 12.3 mg. only for the artificial standards. Even as an "estimation" of the bilirubin content the older method is evidently progressively lower for the higher bilirubin values.

Normal values obtained with our procedure were determined on 7 subjects. The average of this series was 1.5 mg., the minimum 0.8 mg., and the maximum 2.0 mg. We have, however, obtained normals of 0.1 mg. to 0.5 mg. As in the other van den Bergh methods, values less than 2 mg. give a weak reaction and are difficult to compare in the colorimeter.

Values for plasma bilirubin in various pathological conditions have been selected for Table I.

TABLE I.
Plasma Bilirubin in Pathologic Bilirubinemia.

Diagnosis	Type of Reaction	Bilirubin mg. p.c.
1. Cardiac failure, mitral stenosis	Indirect	7.2
2. Pernicious anemia, 6 patients	”	1.5-6.3
3. Congenital hemolytic icterus, 3 pat.	”	4.0-5.2
after splenectomy, 2 patients	”	0.1-1.5
4. Acquired hemolytic icterus	”	7.2
5. Acute arsenical intoxication, lues	Direct	5.6-17.5
6. Catarrhal jaundice	”	49.2
7. Common duct stone, 2 patients	”	23.5-30.0
after operation, 1 patient	”	8.0
8. Obstructive jaundice, carcinoma	”	14.0
9. Chronic cholocystitis	”	10.6
10. Syphilis, hepatic	Indirect	2.0
11. Cirrhosis of the liver	”	2.4
12. Splenomegaly, etiology ?	”	0.5
13. Toxemia of pregnancy, 2 patients	”	1.6-3.0

7149

Phosphorus Partition in Blood and Serum, Serum Calcium and Plasma Phosphatase during Healing of Late Rickets.

GENEVIEVE STEARNS AND EDNA WARWEG. (Introduced by P. C. Jeans.)

From the Department of Pediatrics, State University of Iowa.

The partition of phosphorus in whole blood and serum, the serum calcium and plasma phosphatase have been studied in 2 patients during the slow healing of late rickets of the low phosphorus type. The evidence obtained indicates that the organic acid-soluble (ester) phosphorus of corpuscles is markedly decreased in active late rickets. The lowest value observed was 41.5 mg. per 100 cc. (normal for the age, 66 mg.). This fraction of the blood phosphorus responds very quickly when antirachitic treatment is given, and may reach the normal value before any definite change in serum inorganic phosphorus is observed. The intake of vitamin D by one patient was apparently insufficient to maintain the increased level of corpuscle ester phosphorus. Relatively much greater increases in vitamin D intake were necessary to induce a second rise, and the normal level was not attained until after the serum inorganic phosphorus had been normal for a period of about 4 weeks.

The plasma phosphatase values of both patients were 5 to 6 times the normal at the beginning of treatment. The amounts decreased during healing but were still high when roentgenological healing was complete. In one of the subjects, the phosphatase remained at double the normal value for more than a year after complete healing occurred.

7150 C

Creatine Content of the Heart in Experimental Cardiac Hypertrophy.

D. W. COWAN. (Introduced by H. M. Hines.)

From the Department of Physiology, State University of Iowa.

It was shown[1] that the concentration of creatine in the human myocardium parallels the heart's ability to maintain an adequate circulation. It follows that if, as is commonly supposed, hypertrophy increases the power of cardiac muscle to do work, the total amount of creatine in a hypertrophied heart should exceed the amount found in one of normal size.

Cardiac hypertrophy was produced in growing rats by the production of nutritional anemia as described by Forman and Daniels.[2] A litter mate of each diet rat was maintained on a standard ration of rat biscuits and water. Occasional bleeding of the diet rats (for hemoglobin estimation) doubtlessly hastened the production of anemia in these animals.

At the end of from 66 to 75 days the anemic rats, together with their litter mate controls, were killed by a blow on the head followed by section of the neck vessels. Hemoglobin estimations were done with a Sahli hemoglobinometer on samples of blood thus collected. Each rat was opened immediately after death, and the ventricles severed from the body by careful section through the atrioventricular ring. The ventricles were then opened, blotted between filters to remove all free blood, weighed, and analyzed for creatine by the method of Rose, Helmer, and Chanutin.[3] A second group of controls was picked from stock, each animal of this series weighing within 5 gm. of the anemic rat with which he was compared. This size control group was necessary since the anemic rats did not maintain normal growth curves. This group also allowed comparison of creatine concentration at different ages, since they were younger animals. There were 11 anemic rats, 10 litter mate controls, and 11 size controls. The results are shown in Table I.

The marked hypertrophy produced by the diet is shown by the fact that the actual ventricular weight averaged 36% higher in the anemic rats than in the litter mate controls, although the former

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[1] Cowan, D. W., *Am. Heart J.*, in press.

[2] Forman, M. B., and Daniels, A. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 479.

[3] Rose, W. C., Helmer, O. M., and Chanutin, A., *J. Biol. Chem.*, 1927, **75**, 543.

TABLE I.

Data comparison of anemic rats with litter mate controls and with size controls. Each value is followed by its standard deviation.

	Litter mate controls	Anemic rats	Size controls
Hemoglobin, %	17.9 ± 1.	3.7 ± 1.7	
Ventricular wt., mg.	665.7 ± 87.2	904.8 ± 120.3	423.5 ± 95.
Body wt., gm.	228. ± 33.	120. ± 37.	122. ± 34.
Ventricular wt./body wt. ratio	2.93 ± 0.22	8.27 ± 2.60	3.51 ± 0.25
Creatine in ventricles, mg. %	192. ± 17.	141. ± 17.	206. ± 11.
Total creatine in ventricles, mg.	1.27 ± 0.15	1.27 ± 0.17	0.87 ± 0.19
Total creatine in ventricles, mg./100 gm. of body wt.	0.56 ± 0.04	1.13 ± 0.28	0.72 ± 0.05

had body weights which averaged 47% below normal. It will be noted that the ratio of ventricular weight (mg.) to body weight (gm.) is normally larger in smaller rats, so that when using this ratio as an index to the degree of hypertrophy, the size factor must be taken into consideration.

The average creatine concentration of the hypertrophied ventricles was 26.6% lower than that of the litter mate controls. However, the total amount of creatine in the ventricles was the same for these 2 series. The data show that normal growth hypertrophy is accompanied by a proportionate addition of creatine; the total amount of cardiac creatine increases with increased muscle mass, thus keeping the concentration approximately constant. On the other hand, in the anemic rats the creatine was added to the heart at the normal growth rate, while the muscle mass increased much more rapidly. Therefore, there seems to be a chemical difference between normal growth hypertrophy and hypertrophy caused by the anemia producing diet. It may be concluded that there is no more creatine in the hypertrophied ventricles of an anemic growing rat than there is in those of a normal rat of the same age.

It is to be admitted that dietary factors other than those concerned with the production of anemia may have had something to do with the lack of creatine in the excess tissue. It is also probable that this hypertrophy is not comparable in all respects to other types of so-called "compensatory" hypertrophy.

7151 P

Appearance of Accessory "Organizers" in Overripe Eggs of the Frog.*

EMIL WITSCHI.

From the Zoological Laboratory, State University of Iowa.

The author has described[1] the condition of overripeness that develops in frog eggs if fertilization is delayed 3 to 5 days after ovulation. Among the abnormalities of embryonic development that follow, one of the most striking is the frequent appearance of reduplications. In the early gastrulation stage instead of the normal upper blastopore lip of limited extent and regular curvature there often appear long and irregular clefts and folds in the equatorial region. Attempts at gastrulation become visible not only on one side but all around the whole circumference of the embryo. As time progresses multiple blastopores and multiple gastrulation lead to the development of twin embryos. As we have pointed out previously,[1] this effect indicates that overripeness has brought about 2 concomitant changes in the embryos. First, the physiological correlations by which an embryo develops as a balanced organism are impaired. These correlations largely rest on inhibitory functions by which established "organizers" (inductors) suppress either the formation or the effective functioning of competing organizers. Secondly, "organizers" appear in lateral and ventral regions of embryos, where normally they never occur. We have described this as self-differentiation or merogenesis of physiologically isolated parts. The mechanism of this formation of secondary organizers remained a complete puzzle until the recent discovery of Holtfreter[2] that material from the ventral region of an early embryo which has no inductive qualities in living condition, acquires such potencies after being killed by heat, dryness, etc. We are inclined now to assume that the possibility to develop into organizer is inherent to any part of the embryo, probably even to every cell. The inhibitors that suppress their realization are destroyed by such factors as heat and dryness but also by the process of overripeness.

The accessory organizers induce characteristically different extra-embryos according to size and more lateral or ventral position.

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* Aided by grants from the Elizabeth Thompson Science Fund.

[1] Witschi, Emil, *Verh. Natf. Ges. Basel*, 1922, **34**, 33; *Verh. Deutsche Zool. Ges.*, 1925, **30**, 91; PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 475.

[2] Holtfreter, J., *Naturwissenschaften*, 1933, **21**, 766.

Mainly, however, is the final result determined by the degree of invagination during gastrulation. If an organizer is fully invaginated its apical end comes to lie near the animal pole of the egg and therefore in close proximity to the corresponding anterior end of the normal organizer. Ten cases of this type have been investigated. In every one a common dorso-ventral plane of symmetry has developed. A common heart is present and on the dorsal side the 2 neural tubes fuse either already in the cervical region or at least at the root of the common tail. The notochords always show the largest extent of duplication, often until near the end of the tail.

More interesting are over 20 cases of incomplete invagination. The anterior end may be found anywhere from the closing blastopore up to near the anterior pole. With the exception of the most rudimentary cases a separate heart forms anterior to the neural cord of the secondary embryo. A notochord of normal diameter is only present in embryos that possess a complete entodermal foregut, complete rhombencephalon, 2 ear vesicles and at least fragments of the mesencephalon. In several cases where the anterior pharynx is incomplete and only one ear vesicle present a notochord of reduced diameter has formed. If no ear vesicles are present and the foregut is more rudimentary, no notochord is ever found. This relationship is of interest because it proves that presumptive notochord is not definitely determined as notochord inductor unless it enters into certain relationships with other fields of differentiation near the anterior pole. From here the notochord inductor travels posteriorly like a rocket, leaving in its trail determined and immediately differentiating notochord material.

Unlike the notochord the neural primordium can start at any point. If invagination is very incomplete the secondary organizer becomes imbedded in the tailbud and may be carried subsequently back into the tail where merely a piece of tail-neural-tube is found, accompanied by some muscle fibers and an extra tail fin. Secondary neural tubes and secondary notochords may finally fuse with the corresponding primary structure though more often they remain completely separate. The diameter of the neural tube has no influence on the diameter of the notochord, and the fact that neural tubes in many instances develop in the absence of notochords proves that they are products of independent induction.

Pacific Coast Section.

December 13, 1933.

7152 C

An Attempt to Reactivate Mercuric Bichloride Inactivated Vaccinia Virus.

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A property common to many enzymes[1, 2] is the ease with which mercury bichloride inactivation may be quantitatively reversed. Such reactivation after bichloride inactivation has also been demonstrated with bacteriophage[3, 4] and since some workers have postulated an analogy between phage and viruses the following experiments were undertaken to determine whether or not bichloride inactivated vaccinia virus could be reactivated.

The least amount of mercury bichloride which would extinguish the reaction of the viruses in rabbit skin was first determined. A calf-skin strain (dermovaccine) and a rabbit-brain strain (neurolapine) of virus were used. Heated and unheated virus-saline mixtures were used as controls. Portions of a 1-500 suspension of the viruses (known to be active in a 1-1,000 suspension) were exposed to equal amounts of various mercury bichloride dilutions for 24 hours at room temperature. One-tenth cubic centimeter of each virus-bichloride or virus-saline mixture was injected intradermally into corresponding shaved skin areas on 2 albino rabbits. The heated virus-saline or heated virus-bichloride mixtures caused no reaction on the skin of rabbits. The reaction of the dermovaccine was extinguished by the 1-10,000 dilution but not by the 1-100,000 dilution of bichloride. The reaction of the neurolapine was ex-

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[1] Marston, H. R., *Biochem. J.*, 1923, **17**, 851.

[2] Euler, H. V., and Svanberg, O., *Fermentforsch.*, 1919, **3**, 330; 1920, **4**, 29, 90, 142.

[3] Krueger, A. P., and Baldwin, D. M., *J. Gen. Physiol.*, 1933, **17**, 129.

[4] Krueger, A. P., and Baldwin, D. M., *J. Gen. Physiol.*, in press.

tinguished by a 1-1,000 but not by any higher dilution of bichloride.

The specific doses of bichloride were applied to fresh suspensions of the viruses and half of the mixtures treated with H_2S, which treatment had restored the activity of bacteriophage. Virus-saline mixtures treated with H_2S served as additional controls. The experiment was performed twice. In each instance the addition of H_2S to the virus-bichloride mixtures did not render the mixture active when inoculated into rabbit skin. The addition of H_2S to virus-saline mixtures did not destroy the virus though there was slight diminution in the extent and severity of the reaction in rabbit skin. This was probably due to unfavorable changes in the tonicity and acidity of the suspending medium.

7153 P

Effect of a Diet Consisting Exclusively of Carrots on Serum Protein Concentration of the Rat.*

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From the Department of Medicine, Stanford University Medical School.

It was pointed out[1] that no significant fall of serum protein concentration occurred in rats kept for a period of 5 months on a diet extremely low in protein, provided the diet was otherwise adequate. These results are at variance with the conclusions of Kohman,[2] and of Frisch, Mendel and Peters.[3] However, the diet used by these observers consisted in large part of carrots and the question therefore came up whether carrots alone may exercise some specific blood protein lowering effect. The present experiments were planned to obtain information about this possibility.

Eighty-eight young adult white rats were placed on a diet consisting exclusively of fresh carrots. No salts or vitamins were added. After varying intervals groups of 5 or 6 animals were exsanguinated under ether anesthesia, aliquot portions of serum were pooled and the total serum proteins were measured.[4] The experiment lasted 145 days during which time the rats remained well and active and there were none of the usual signs of vitamin deficiency. There was progressive weight loss.

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* Supported, in part, by a grant from the Rockefeller Fluid Research Fund of the School of Medicine, Stanford University.

[1] Bloomfield, A. L., *J. Exp. Med.*, 1933, **57**, 705.

[2] Kohman, E. A., *Am. J. Physiol.*, 1920, **51**, 378.

[3] Frisch, R. A., Mendel, L. B., and Peters, J. P., *J. Biol. Chem.*, 1929, **84**, 167.

[4] Barnett, C. W., Jones, R. B., and Cohn, R., *J. Exp. Med.*, 1932, **55**, 683.

In the majority of the animals there was a definite fall in serum protein concentration and values as low as 4.28, 4.44, 4.72 were obtained with some of the groups (control level 6.30-6.50 gm. %). Five rats among the whole group of 88 developed gross watery effusions into the serous cavities and in one of these animals the serum protein concentration was only 2.99 gm. %. It is of interest that the serum protein level bore no close relation to the *duration* of carrot feeding. Serum protein from a group of rats on the 61st day averaged 4.44 gm. %, from another group on the 82d day 5.06 gm. % and from the final group on the 145th day 4.28 gm. %.

The mechanism of the lowering of serum protein concentration after carrot feeding appears to be extremely complex. Neither lack of protein alone nor lack of vitamins seem to offer a complete explanation.

7154 C

Absence of Compensatory Hypertrophy of Cowper's Glands in the Albino Rat.

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Compensatory hypertrophy may follow a deficiency in the total cell mass induced by operative removal of one of the paired organs. The process is manifested in some but not in all paired organs. The present study was undertaken because no data are available with respect to compensatory hypertrophy in Cowper's glands.

Albino rats of 250 days of age were used. The age of the rat excluded the possible influence of growth on the size of the gland. The stimulus to compensatory hypertrophy was given by the removal of one Cowper's gland from each rat. Under ether anesthesia and through the perineal route, alternate glands were removed from each successive rat. Immediately after removal each gland was freed from any adherent fat or tissue and weighed to within a milligram on a torsion balance. The weights of these glands were taken as controls.

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* Peiping Union Medical College Fellow.

Twenty days was arbitrarily chosen as the period of time during which to determine the ability of the remaining Cowper's gland to undergo compensatory hypertrophy. At the end of this period the rats were etherized and the remaining Cowper's glands removed and carefully weighed as before.

The results are given in Table I. These data show that Cowper's glands do not possess the capacity for compensatory hypertrophy which has been demonstrated in some other organs.

TABLE I.

Control Wt. of removed Cowper's gland at time of operation						Experimental Wt. of remaining Cowper's gland 20 days after operation					
Rat No.	Right gland	Wt. of C. G. per 100 sq. cm. body surface	Rat No.	Left gland	Wt. of C. G. per 100 sq. cm. body surface	Rat No.	Right gland	Wt. of C. G. per 100 sq. cm. body surface	Rat No.	Left gland	Wt. of C. G. per 100 sq. cm. body surface
1	29	5.0	2	30	5.3	2	26	4.6	1	28	4.8
3	26	4.9	4	25	4.2	4	30	5.0	3	28	5.3
5	27	5.5	6	22	4.2	6	15	2.8	5	30	6.1
7	28	4.4	8	34	5.8	8	30	5.1	7	30	4.8
9	28	5.1	10	32	5.2	10	29	6.3	9	28	5.1
11	42	7.0	12	32	5.5	12	28	4.8	11	31	5.2
13	34	5.9	14	31	5.0	14	28	4.6	13	40	7.0
15	32	5.8	16	34	6.2	16	36	6.5	15	32	5.8
17	37	5.8	18	28	5.1	18	28	5.1	17	30	5.5
19	37	6.9	20	36	6.8	20	39	7.3	19	37	6.9
21	34	5.7	22	43	7.9	22	39	7.2	21	30	5.0
23	34	6.0	24	33	5.9	24	32	5.7	23	48	8.5
25	34	6.1	26	42	7.6	26	43	7.8	25	36	6.5
27	38	6.5	28	29	5.7	28	30	5.1	27	44	7.5
29	33	6.2	30	44	7.9	30	29	5.2	29	26	4.9
31	27	4.6	32	24	4.1	32	28	4.8	31	38	6.5
33	24	4.5	34	30	5.7	34	30	5.7	33	25	4.6
35	36	6.9	36	36	6.4	36	28	5.0	35	28	5.3
37	38	7.2	38	28	6.4	38	28	5.1	37	26	5.0
Aver.		5.79			5.84	Aver.		5.46			5.80
								Control			Experimental
Aver. of right and left glands								5.81			5.63
Standard deviation of aver.								0.160			0.158
Probable error of aver.								0.106			0.108
Standard deviation of difference between the two averages									0.151		

7155 C

A Method for Separate Removal of Either Hypophyseal Lobe of the Rat.

GEORGE GIRAGOSSINTZ. (Introduced by E. S. Sundstroem.)

From the Division of Biochemistry, University of California Medical School, Berkeley.

Two methods of approach have been used by investigators for the extirpation of the hypophysis of the rat. The parapharyngeal approach proposed by Smith[1] which has been modified by several investigators is the one most commonly used. The second method, suggested by Koyama,[2] is through the ear. The temporal approach used successfully on dogs by Dandy and Reichert[3] has not been, to our knowledge, used on rats. This method is particularly adapted for operating on rats, (1) because the sella turcica is shallow and a satisfactory approach can be obtained from the side; (2) the rat is very resistant to infection; (3) the anterior and the posterior lobe of the hypophysis in the rat can be removed separately by this approach. This cannot be done with dogs. (4) The lobes can be removed intact and may be used for microscopic examination; (5) the operation can be carried out on very young animals.

The work of Weed and McKibben[4] on the experimental alteration of the brain bulk has been useful in this operation. The decrease in the brain bulk of rats given intravenous injection of hypertonic sodium chloride solution is very striking.

Under light ether anesthesia sufficient hypertonic salt solution is injected intravenously in order to obtain the maximum amount of brain shrinkage. We have been using 400 mg. of sodium chloride per kilo of body weight. This is slightly more than the amount used by Weed and McKibben on cats and by Dandy and Reichert on dogs. In rats weighing over 100 gm., the sodium chloride was injected very slowly into the jugular vein in a 20% solution. Rats weighing less than 100 gm. were given sodium chloride in a 5% solution. If the sodium chloride is injected rapidly, the animal usually goes into convulsions and may die, but when injected very slowly, it has no immediate nor any apparent harmful after effects.

Fifteen minutes after the injection of the salt solution, a small unilateral opening is made with a trephine. The trephine described by Pencharz[5] is satisfactory. The opening is made larger with the aid of a small bone forceps. The zygoma is then removed to give the lowest possible entrance to the base of the brain. The operation can be performed without removing the zygoma, but it is advisable for the beginner to remove it. The dura is then cut and a small flexible spatula, preferably made from collodion, is passed under the temporal lobe of the brain to the region of the hypophysis. The oculomotor nerve interferes with a view of the hypophysis and may be cut. When this has been done very little retraction is now necessary in order to obtain a good view of the hypophysis. With the hypophysis exposed the diaphragma sellae is cut. For this purpose we have made from the point of a needle a very small sickle which was fused to the tip of a small glass rod. Another glass rod drawn so that the tip is very fine and curved is used for separating the posterior from the anterior lobe. One will find that the posterior lobe can be easily separated from the anterior lobe and removed intact. The two lobes may be removed if it is so desired.

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[1] Smith, P. E., *Am. J. Anat.*, 1930, **45**, 205.

[2] Koyama, R., *Jap. J. Med. Sci.*, IV, Pharmacology, 1931, **5**, 41.

[3] Dandy, W. E., and Reichert, F. L., *Bull. Johns Hopkins Hospital*, 1925, **37**, 1.

[4] Weed, L. H., and McKibben, P. S., *Am. J. Physiol.*, 1919, **48**, 531.

Dandy, Reichert and others believe that the large mortality reported by earlier investigators from hypophysectomy on dogs and cats was due to trauma and hemorrhage. We have not been able to observe any ill effects from slight trauma to the brain from this operation insofar as the rat is concerned.

7156 C

Hematopoietic Reactions to Antimonyl Antimony.*

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From the Department of Medicine, University of California Medical School, San Francisco, California.

The close chemical relationship between arsenic and antimony suggests that the latter substance might be used in place of arsenic in the treatment of abnormal leucocytoses. Meneghetti[1] concluded that a "true hemopathy" characterized by leucocytosis followed by leucopenia, a profound anemia, and marked normoblastosis could be produced after a single intravenous inoculation of colloidal antimony

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[5] Pencharz, R., Thesis, University of California Library, 1932.

* Assisted by a grant from the Christine Breon Fund.

[1] Meneghetti, E., *Haematologica*, 1926, 7, 1.

sulfide. His experiments with tartar emetic, however, were incomplete. In a series of preliminary experiments using a freshly prepared 1% solution of potassium antimonyl tartrate we established a dose of 6 mg. per kilo bodyweight as the maximum tolerated initial dose for rabbits. When inoculated intravenously this dose produced a characteristic leucopenia without subsequent leucocytosis.

Normal adult rabbits were inoculated in the marginal ear vein with a freshly prepared 1% solution of potassium antimonyl tartrate. A complete blood count was done before and after each experiment. The total leucocyte and differential counts were done at intervals of one-half, one, 2, 4, 7, and 28 hours after inoculation. The blood films were stained with Wright's stain, and the platelet counts were done using Rees and Ecker's diluting fluid. After the completion of each experiment the animals were sacrificed and a histological study of the hematopoietic organs was made.

The results of 25 experiments using 9 animals are averaged in Table I. The corrected leucocyte and differential counts are given in absolute numbers of cells per cubic millimeter. It was found unnecessary further to analyze these data statistically, because there was very little variation between individual experiments.

TABLE I.
Hematopoietic Reactions after the Intravenous Inoculation of Potassium Antimonyl Tartrate.

	Before inoculation	Hours after inoculation ½	1	2	4	7	28
Hemoglobin % (Sahli)	80						85
R. B. C.'s (millions)	5.90						5.98
Total Leucocytes*	8940	3636	3602	4581	5511	7008	8969
P. M. N. Neutrophiles	3646	857	784	1710	3375	5053	4439
Filamentous	2734	676	613	1126	2150	3034	2985
Non-filamentous	908	181	171	584	1225	2019	1454
Lymphocytes, small	1785	980	1103	1159	687	640	1978
,, large	1964	1007	990	848	730	700	1531
Monocytes	679	247	220	265	220	184	282
Eosinophiles	75	15	17	25	37	38	53
Basophiles	481	303	246	358	363	181	323
D-Cells	0	12	22	75	51	16	25
Smudges	337	212	220	142	148	234	341
Normoblasts	32	41	79	115	232	462	222
Platelets (thousands)†	513	599	500	464	536	518	543

* Leucocyte counts corrected for normoblasts.
† Platelet counts represent an average of 7 experiments.

Within 5 minutes after inoculation it was noted that the leucocyte count was reduced to about one-half of the normal and that the greatest depression occurred about one hour after inoculation. There is an initial granulopenia affecting the filamentous and non-filamentous polymorphonuclear neutrophiles equally. During recovery there is a marked increase in the non-filamentous neutrophiles. There is a latent lymphopenia which reaches its maximum about 7 hours after inoculation. The monocytes are depressed and do not return to normal during the period of the experiment. A peculiar cell with pyknotic nucleus and intensely basophilic staining cytoplasm appears in about one-half hour, and reaches its maximum concentration approximately 2 hours after inoculation. This cell has been designated as the "D-Cell". It probably represents a degeneration form. There is noted a progressive increase in normoblasts which reaches a maximum after 7 hours. These are still present in appreciable numbers after 28 hours.

Summary. Potassium antimonyl tartrate produces a characteristic leucopenia without subsequent leucocytosis when administered intravenously to normal rabbits. It also produces a moderate normoblastic response. It does not affect the platelet count. It is suggested that antimony, like arsenic, might be used to depress abnormal leucocytoses of myeloid cells.

7157 C

In vitro Leprocidal Activity of Some Non-chaulmoogryl Compounds.*

G. A. EMERSON AND A. J. SALLE. (Introduced by C. D. Leake.)

From the Pharmacological Laboratory, University of California Medical School, San Francisco, and the Department of Bacteriology, University of California, Berkeley.

Little emphasis has been placed upon the *in vitro* bactericidal activity against *Mycobacterium leprae* of compounds other than the fixed and essential oils and various fatty acids examined by Walker and Sweeney, Schöbl, and Adams and his co-workers.[1] In view of the recent interest in the clinical use of non-chaulmoogryl compounds, particularly certain dyes, as antileprosy drugs, a report of our accumulated findings on the action of these and other agents against *M. leprae* is made herewith. These observations have been made during the past year as a tentative guide to therapeutic efforts on experimentally infected leprous rats using a standardized technique previously described.[2]

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* Part of a cooperative study of the chemotherapy of leprosy conducted by the Hooper Foundation for Medical Research and the Pharmacological Laboratory of the University of California Medical School, San Francisco, and supported in part by the Christine Breon Fund for Medical Research.

[1] Walker, E. L., and Sweeney, M. A., *J. Inf. Dis.*, 1920, **26**, 238; Schöbl, O., *Phil. J. Sci.*, 1923, **23**, 533; *Ibid.*, 1924, **24**, 23; Stanley, W. M., Coleman, G. H., Greer, C. M., Sacks, J., and Adams, R., *J Pharmacol. Exp. Therap.*, 1932, **45**, 121.

The leprocidal activity of over 200 chemical agents falling into 4 general types has been studied by the 2 appropriate techniques described by Adams *et al.*,[1] with careful adherence to the precautions noted by these authors. A single strain of test organism was used, this being *M. leprae hominis*, Mary Puhulahula strain, isolated by Dr. E. L. Walker.† The media used as substrate in the tests was that of Long and Seibert,[3] recommended for our use by Dr. Walker, rather than a 5% glycerol broth as used by Adams[1] in his tests. No other variations from Adams' method were made. Most of the tests were carried out in Dr. Walker's laboratory and in conjunction with the Bacteriological Laboratory of the University of California. By closely following the bacteriological technique worked out by Adams, his estimate of a probable error of ±10% appears justified.

The first group of agents studied was a series of dyes. The magnitude of the effects with the more potent of these is noted in Table I. Among those found ineffective, a few are of special interest and deserve comment.

Trypan blue was found to be ineffective in solutions of less than 0.1%, a concentration which will be referred to as "ineffective" in this paper since from 50-75 gm. of the agent in question would be required to furnish this concentration in the body even if no segregation occurred. Since Ryrie[4] has reported trypan blue to be of value when given intravenously in human lepers in much smaller doses (1.0 gm.), it is not probable that trypan blue acts therapeutically by virtue of its bactericidal action even though some concentration of the dye may take place in the lepromata, as reported.[4] In regard to this selective absorption of trypan blue by the leproma, a similar phenomenon was noted in leprous rats by Oliver,[5] who further observed in sections of the tissues that only the healthy macro-

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[2] Anderson, H. H., Emerson, G. A., and Leake, C. D., *Internat. J. Leprosy*, in press.

† Whether or not this organism is the pathogen of leprosy does not concern us here. Adams has shown the susceptibility of different strains of *M. leprae* to various drugs to be the same.

[3] Long, E. R., and Seibert, F. B., *Am. Rev. Tuberc.*, 1926, **13**, 393.

[4] Ryrie, G. A., *Trans. Royal Soc. Trop. Med. and Hyg.*, 1933, **27**, 85.

[5] Oliver, J., *J. Exp. Med.*, 1926, **43**, 233.

TABLE I.

Minimal Certain Leprocidal Concentration of Various Agents in Long's Media.*

Substance	pK_{lep}†	Substance	pK_{lep}†
Crystal violet	5.7	Merthiolate	6.3
Methyl violet	5.7	Metaphen	6.0
Brilliant green	5.7	Mercurochrome	5.7
Malachite green	4.7	Solganol	5.3
Methylene blue	5.3	Cu morrhuate	5.0
Safranin	5.1	Ag proteinate	5.0
Toluidin blue	5.1	Fuadin	4.7
Thionin	5.1	Arsphenamine	4.7
Pyronin	5.1	Emetine Bismuth iodide	5.0
Rose bengal	4.0	Emetine Sb iodide	5.0
Octyl resorcinol	5.3	Isoamyl cephaelin phos.	4.7
Heptyl resorcinol	5.0	Yatren	4.3
Hexyl resorcinol	5.0	Vanillin	5.0
Na glycocholate	4.7	Na thioglycolate	5.0
Na taurocholate	4.7	Thymol	4.0
Na F	4.3	Carvacrol	4.0
Salicyl aldehyde	4.3	Eugenol	4.0

* Single strain of *M. leprae* used throughout, against which Na hydnocarpate is active in concentration of 1:40,000, *i. e.*, $pK_{lep} = 4.6$.

† pK_{lep} = neg. log concentration in gm./cc.

phages about the lesion stored the dye in quantity, while the infected so-called "lepra cells" took up smaller amounts in inverse proportion to their content of lepra bacilli.

Eosin and fluorescein, found by Ryrie to be unsuited to leprosy treatment, were found to be without leprocidal activity, while mercurochrome (dibromoxymercurifluorescein) used intravenously with success by Denney,[6] has a high activity (Table I). Acriflavine, used clinically with poor results,[7] is active only in concentrations above 0.01%. Other ineffective dyes were: alizarin, tropaoelin 00, indigo, methyl orange, carmine, Bismarck brown, methyl red, purpurine, vital red, bromcresol green, congo red, azolitmin and rosaniline. Neutral red and rose bengal inhibited growth in concentration of 0.01%. Selective absorption of the dye from solution by the growing colonies was noted chiefly in the case of congo red, rose bengal, and brilliant vital red.

The simpler phthalein dyes were also found ineffective, which is of interest since soaps of fatty acids assayed for leprocidal activity by Adams' technique are necessarily contaminated with a small amount of phenolphthalein. Halogenation does not increase their activity, since tetraiodo-, tetrabrom-, and tetrachlor-phenolphthaleins are ineffective also. Phenolsulphonephthalein and thymolsulphonephthalein are ineffective, while o-cresolsulphonephthalein is active in concentrations of 0.01%. Again tetrabromphenolsulphonephthalein, dibromthymolsulphonephthalein and dibromcresolsulphonephthalein are not active

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[6] Denney, O. E., *Pub. Health Rep.*, 1931, **46**, 5.

[7] Schwetz, J., *Ann. Soc. Belge de Med. Trop.*, 1933, **13**, 43.

The *second* group of substances studied were metallic organic compounds most of which have been used with varying success on human leprous subjects.[8] The more potent leprocidal drugs of this group are represented in Table I, where it may be noted that Hg, Sb, Au, Ag and Cu organic compounds have high activity. Pentavalent arsenical compounds were without effect, atoxyl, carbarsone, thiocarbaminophenyl arsonic acid, acetarsone, etharsenol, proparsenol and tryparsamide being taken as examples. Activity among the trivalent arsenicals is variable; while arsphenamine is highly active, arsenious trithiosalicylic acid and arsenious trithiophenylsulfonic acid are ineffective. Na or K iodides were active only in a concentration of 1%, as is iodoform also.

The *third* group includes a miscellaneous series selected for properties which in the chaulmoogric acid derivatives have been held to cause the peculiar activity of the molecule. Surface tension lowering agents, enzymes, highly unsaturated compounds, compounds containing 16-18 carbon atoms, specific and general protoplasmic poisons were tested. The highly effective compounds in this series may be found in Table I, while some of the more interesting of the ineffective compounds were: papain, saponin, p-phenylenediamine, pyridine, benzaldehyde, p-dimethylaminobenzaldehyde, cuprous cyanide, guaiacol, aniline, dimethyl aniline, diphenyl amine, benzidine, hydroxylamine HCl, Bayer 205, ammonium iodoxybenzoate, hydroquinone, monobromcamphor, menthol, emetine, quinine dihydrochloride, optochin, berberine, cinchophen, vioform and acetonitrile. Alcohols were particularly ineffective, for growth occurred in 10% solutions of methanol, while growth in 1% solutions occurred in the series up to iso-butanol, and in 0.1% up to capryl. Allyl alcohol and tribromethanol also were inactive. It appears that in some respects ethanol is a poor antiseptic for use in leprosaria. While resorcinol itself was inactive, hexyl to octyl resorcinols were markedly effective, as shown in Table I.

A *fourth* group of compounds considered were certain fatty acids from sources other than chaulmoogra. Our findings on the action of 13 chaulmoogric acid derivatives have been reported elsewhere.[9] The plating method was used to evaluate agents of this group, and the tabulated results in Table II represent the mean diameter of the circle of inhibited growth about a 0.05 cc. drop of the test substance placed in the center of an inoculated 15 cc. plated dish of 5% glycerol agar, after 144 hours of growth. In regard to ricinoleic acid,‡ it may be noted that Adams[1] found inhibition in dilutions below 0.002% for "total castor oil acids" but nevertheless considered them inactive.

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[8] Eubanas, F. C., *J. Philipp. Is. Med. Ass.*, 1927, **7**, 319; Hoffmann, W. H., *Arch. f. Schiffs.-u. Tropen Hyg.*, 1927, **31**, 139; Paldrock, A., *Arch. f. Schiffs.-u. Tropen Hyg.*, 1927, **31**, 459; *Ibid.*, 1932, **36**, 136; *Dermatol. Wochenschr.*, 1931, **92**, 273; Feldt, A., *Klin. Wochenschr.*, 1928, **7**, 73; Palmer, F. J., *Brit. Med. J.*, 1925, **2**, 96; Koga, G., *Jap. Med. Lit.*, 1917, **2**:**3**, 4.

TABLE II.
Leprocidal Activity of Some Fatty Acids and Related Compounds by a Plating Technique.

Test Substance	Diameter of Inhibited Area
	cm.
Total Hydnocarpus glycerides	2.0
,, Chaulmoogra glycerides	1.5
,, Calophyllum glycerides	0
,, Castor oil glycerides	0
,, chaulmoogra acids	2.5
Chaulmoogric acid	1.5
Total castor oil acids	3.5
Ricinoleic acid	3.0
Cinnamyl chaulmoograte	1.0
Ethyl chaulmoograte	0
,, clupanodonate	0
,, ricinoleate	4.0
Butyl ricinoleate	0
Methyl ricinoleate	0
,, undecylineate	2.0
Undecylinic acid	2.5
Ethyl acetylricinoleate	0
Acetyl ricinoleic acid	3.0
Ethyl polymerized ricinoleates	3.0
Polymerized ricinoleic acids	4.5
Ethyl acetyl polymerized ricinoleates	0
Acetylated polymerized ricinoleic acids	2.0
"Chaulphosphate"	2.0
"Chaulphosphate" saturated aqueous solution	3.5
Na di-n-heptyl acetate saturated aqueous solution	7.5

Tests of these 4 groups of compounds were made in order to select promising drugs for experimental therapy in leprous rats. At present, several series of infected rats have been under treatment with merthiolate, gadusan, trypan blue, brilliant green, gentian violet and solganol for four months. A study of the toxicity of these compounds, and the outcome of the experimental therapy will be reported shortly.

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[9] Emerson, G. A., Anderson, H. H., and Leake, C. D., *Am. J. Trop. Med.*, in press; PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 274.

‡ The ricinoleates and other castor oil derivatives tested in Table II were generously furnished by Mr. Kendall Marsh of the Baker Castor Oil Co., New York.

Summary. In a survey of the *in vitro* leprocidal effects of some 200 dyes, organic metallic compounds, fatty acids and miscellaneous compounds, high activity was found especially for merthiolate, metaphen, mercurochrome, solganol, triphenylmethane dyes, methylene blue, and octyl resorcinol. Trypan blue and the aliphatic alcohols were surprisingly inactive. While some correlation between therapeutic value and leprocidal activity may be noted, simple bactericidal tests are by no means a satisfactory excuse for clinical trial but are necessarily preliminary to the comprehensive pharmacological evaluation we have recommended.

New York Meeting.

New York Academy of Medicine, January 17, 1934.

7158 P

Absence of Effect of Antuitrin-S Injections on the Immature Ovary.*

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In a report[1] dealing with the effect of Antuitrin-S on the mature human ovary, it was pointed out that the response was not similar to that obtained in the rodent. It was therefore planned to inject a series of immature human females and examine their pelvic organs. Naturally such an opportunity is limited, for the cases must be selected from patients who are suffering from diseases that are of themselves fatal.

We have had opportunity to study 2 cases, one an infant of 10 months and one of 18 months. Both were suffering from tuberculous meningitis, proved by bacteriological examination of the spinal fluid and later confirmed at autopsy.

In the first case a 10 months infant received a total of 2,500 rat units of Antuitrin-S† in 5 divided doses over a period of 96 hours. The injections were given intramuscularly in the buttocks. The last injection was given 12 hours before death. At autopsy the ovaries both macroscopically and microscopically presented no evidence of follicular maturation or luteinization. The uterine mucosa showed no evidence of hypertrophy or cystic glandular hyperplasia. The second case, an infant of 18 months, also received a total of 2,500 rat units of Antuitrin-S. The last injection in this case was 16 hours before death. At autopsy the ovaries both macroscopically and microscopically presented no evidence of follicle maturation or luteinization. One ovary showed a tiny pinpoint area of hemorrhage on the surface. The uterine mucosa did not show any variation from the normal.

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*I wish to thank Doctor Frank Spielman for his assistance.

[1] *Am. J. Obstet. and Gynecol.*, 1933, **26**, 588.

† The material was supplied by Parke-Davis & Co., whom we wish to thank for their cooperation and interest.

Several patients desperately ill with gastro-intestinal and brain tumors received similar or greater amounts, but recovered from their illnesses.

The examination of a complete series of cases up to the age of puberty will of necessity require much time.

Conclusions. 1. Antuitrin-S intramuscularly and intravenously apparently produces no by-effects. 2. Antuitrin-S in 2 cases failed to produce follicular maturation or luteinization in the immature human ovary or changes in the uterine mucosa.

7159 C

A Method of Obtaining Undiluted Tissue Juice for the Study of Tissue Antibody Titer.

BEATRICE CARRIER SEEGAL AND DEVORAH KHORAZO.

From the Department of Bacteriology, and the Department of Ophthalmological Research, College of Physicians and Surgeons, Columbia University, New York.

The problem of obtaining suitable material for the determination of the antibody content of tissues or organs has usually been met by grinding the tissues with sand and extracting in a solvent, either physiological saline alone or equal parts of the saline solution and glycerine. The method is tedious and the material obtained has already undergone dilution which is not always desirable. The ease with which tissue juices are obtained by pressure in certain experiments carried out by Dr. Foster of the Department of Biochemistry suggested to us the possibility of extracting organs by this procedure for use in immunological experiments. The method consists in placing the tissue in an hydraulic press and extracting its fluid content under a pressure of approximately 16,000 pounds per square inch. The method may be used for any organ but is particularly serviceable in extracting skin and subcutaneous tissue where the large amount of connective tissue makes other methods of extraction difficult.

The tissue to be extracted is cut into pieces and mixed with a large volume of portions of filter paper about 1 cm. square. The whole mixture is cut up further until it consists of a grumous mass of filter paper which has absorbed all the bits of tissue to it. The filter paper offers traction and prevents the tissues from sliding out of the press. This mixture of filter paper and tissue is then wrapped in a piece of cheese cloth and is ready to be pressed for the extraction of its fluid content. The press used is an hydraulic press manufactured by Fred S. Carver of New York City, capable of exerting a pressure of 20,000 pounds per square inch. As the press is too large to extract the small amounts of tissue used in our experiments, a small press to fit inside the large one was constructed. This consists of a solid steel cylinder 4.7 cm. in diameter at the base. Midway from the base the cylinder is turned down to a diameter of 4 cm. The whole is enclosed in a brass jacket which extends 1.2 cm. above the steel cylinder and which contains a spout to drain the space between cylinder and jacket at the point where the cylinder is narrowed. Another solid steel cylinder 4 cm. in diameter and 3 cm. high is used as a plunger to exert the pressure from above.* The accompanying diagram illustrates the construction of this press. The packet of tissue to be extracted is placed in this small press and

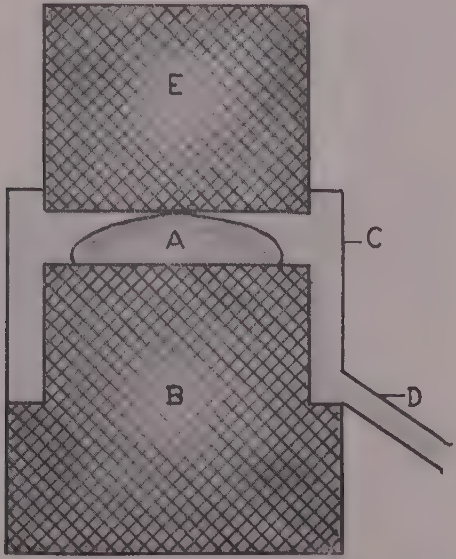

Diagram I. Cross section of small press which is placed inside large press to extract fluid content of tissues. A, Packet of cut up tissue and filter paper. B, Solid steel cylinder. C, Brass jacket. D, Spout to collect fluid. E, Solid steel plunger used to exert the pressure from above.

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* We are indebted to Mr. Rosebury of the Department of Biochemistry for constructing this small press.

the whole is placed in the large press under an average of 16,000 pounds per square inch of pressure. When small pieces of tissue are extracted, about 15% to 20% by weight is recovered as fluid. The juice thus obtained contains considerable fat and is therefore allowed to stand in the icebox overnight. The following morning it is centrifuged in the cold centrifuge† and the clear fluid which lies underneath the fat is removed with a capillary pipette. This extracted fluid is suitable for carrying out either precipitin or agglutinin determinations. When it is to be used for precipitin determinations, repeated centrifuging in the cold centrifuge is usually advisable. Since these organ extracts sometimes precipitate spontaneously when incubated at 37°C., all tests are put in the icebox immediately and are read in 24 hours and again in 48 hours, according to the method of Heidelberger, Kendall and Soo Hoo.[1] The end titers obtained in the icebox are the same as those obtained by a preliminary incubation at 37°C. followed by icebox treatment.

The pressure exerted by this method of extraction is apparently sufficient to break up body cells, for stained films of the residue of dry filter paper and tissue have failed to show any intact cells.

The agglutinin titer of tissue juice obtained by this method has been compared with that obtained by the method of Cary[2] of grinding with sand and extracting with equal parts of physiological saline and glycerine. The titers obtained by the pressure extraction are equal to or higher than those obtained by the method of Cary.

7160 P

Local Organ Hypersensitiveness. VII. Demonstration of Agglutinins in Tissues of the Rabbit Eye Following Immunization with Eb. Typhi Vaccine.

BEATRICE CARRIER SEEGAL AND DAVID SEEGAL.

From the Departments of Bacteriology and Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York.

The injection of a foreign protein into the anterior chamber of the rabbit's eye results in a local sensitization of this organ. Weeks or months later when the eye has returned to a normal appearance the intravenous injection of the same protein produces a marked inflammation of the iris and conjunctiva. The opposite, control eye shows no reaction or only a slight hyperemia.[1] This local hypersensitivity has the characteristics of an antigen-antibody reaction.[1, 2] The presence of antibodies in the tissues of the hypersensitive eye was not demonstrated, however, by the technique previously employed.[2]

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† Manufactured by the International Equipment Company, Boston, Mass.

[1] Heidelberger, M., Kendall, F., and Soo Hoo, C. M., *J. Exp. Med.*, 1933, **58**, 137.

[2] Cary, W. E., *J. Med. Research*, 1922, **43**, 399.

This paper reports further studies on the antibody content of the juices extracted from the tissues of the injected eye. By an alteration in the technique of extracting the tissues and the utilization of a particulate antigen which stimulates the production of agglutinins it has been possible to demonstrate the concentration of antibody in the tissues of an eye injected with typhoid vaccine.

A series of 12 adult albino rabbits were injected into the anterior chamber of the right eye with 0.1 or 0.15 cc. of New York City Board of Health typhoid vaccine which contained approximately 3 billion organisms per cc. The injection was given under cocaine anesthesia by the method already described.[1] The animals were sacrificed 4 to 102 days later by bleeding from the heart. Sixty to 120 cc. of blood was obtained. The anterior chamber fluids were removed and the palpebral and scleral conjunctiva together with the tissues from the anterior two-thirds of the eyeball from each eye were dissected out. The lens was expelled and the excess aqueous and vitreous humors were blotted off. The tissue juice from these fragments of tissues, consisting of conjunctiva, iris, sclera and cornea, was extracted with pressure by a method previously described.*[3] The fluids so obtained were tested for their agglutinin titer and the titers compared with those obtained in the serum and the aqueous humors. A series of 11 control animals was similarly tested. The treatment of the controls is indicated in Table I.

The results of the experiments are summarized in Table I. In those animals injected with the typhoid vaccine in the anterior chamber of the eye, agglutinins appeared first in the serum. During the first month following the injection of the vaccine the serum

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[1] Seegal, D., and Seegal, B. C., *J. Exp. Med.*, 1931, **54**, 249.

[2] Seegal, B. C., Seegal, D., and Khorazo, D., *J. Immun.*, 1933, **25**, 207.

* When the animal has been exsanguinated there can be only a trace of blood in this juice. The relative absence of blood is indicated by the fact that the tissue extract is not colored with hemoglobin which is the case with the fluid extracted from an organ which contains gross blood.

[3] Seegal, B. C., The accompanying paper on "A Method of Obtaining Undiluted Tissue Juice for the Study of Tissue Antibody Titer."

TABLE I.

Typhoid agglutinin titer of serum, anterior chamber fluids and extracted tissues of the eye following the injection of a vaccine of *Eb. typhi* into the anterior chamber of the right eye; also similar tests in a series of control animals.

				Agglutinin Titer in						
Animal No.	Preliminary treatment	Place of injection	Days	Control sample serum	Control Sample Rt. ant. ch. fl.	Serum	Rt. ant. ch. fl.	Lt. ant. ch. fl.	Tissue juices right eye	Tissue juices left eye
515	Typhoid vac.	Right ant. chamber	4	0	0	1-40	0	0	0	0
513	,, ,,	,, ,, ,,	6	0	0	1-64	0	0	1-8	0
516	,, ,,	,, ,, ,,	8	0	0	1-512	1-16	0	1-16	0
514	,, ,,	,, ,, ,,	12	0	0	1-1024	1-512	1-8	1-256	1-64
227	,, ,,	,, ,, ,,	35	—	0	1-320	1-80	0	1-160	1-40
228	,, ,,	,, ,, ,,	37	—	0	1-1280	1-20	0	1-640	1-320
511	,, ,,	,, ,, ,,	46	0	0	1-1024	1-128	0	1-2920	1-64
231	,, ,,	,, ,, ,,	49	—	—	1-160	1-80	0	1-320	1-40
1310	,, ,,	,, ,, ,,	50	1-16	0	1-512	1-512	0	1-2048	1-16
229	,, ,,	,, ,, ,,	84	—	0	1-64	0	0	1-64	1-8
230	,, ,,	,, ,, ,,	98	—	—	1-64	1-64	0	1-256	1-16
512	,, ,,	,, ,, ,,	102	0	0	1-640	1-32	0	1-2048	1-64
1	None	—	—	—	—	—	—	—	0	0
2	,,	—	—	—	—	0	0	0	0	0
3	,,	—	—	—	—	0	0	0	0	0
1312	,,	—	—	—	—	1-512	0	0	1-16	1-16
583	Horse serum	Subcut.	30			1-16	0	0	0	0
584	,, ,,	,,	32	—	—	0	0	0	0	0
2521	Typhoid vac.	,,	8	—	—	1-512	0	0	1-16	1-16
1615	,, ,,	Intraven.	10	0	—	1-4000	1-16	1-32	1-1000	1-1000
1627	,, ,,	,,	19	0	—	1-8000	1-32	1-32	1-2000	1-2000
4	,, ,,	Subcut.	60	—	—	1-160	1-6	1-6	1-40	1-40
2603	,, ,,	Intraven.	240	—	—	1-800	1-8	1-8	1-256	1-256

agglutinin titer was higher than that of any of the other fluids tested. The titer of the anterior chamber fluid of the injected eye was variable but never higher than that of the serum. Agglutinins failed to appear in the anterior chamber fluid of the uninjected eye.† The tissue juices extracted from the iris, conjunctiva and sclera of the injected eye gave a titer of agglutinins which surpassed that found in the tissue juices of the normal eye but for the first month this titer did not surpass that of the serum. In those animals sacrificed from the 46th to the 102nd day, however, the antibody titer of the tissue juices extracted from the injected eye was equal to or greater than that of the serum. It also surpassed the antibody titer of the noninjected control eye 8 to 128 times. These results indicate that there is a concentration of specific agglutinins in the fluids of the tissues surrounding the anterior chamber of an eye which has been injected with typhoid vaccine. The data do not answer the questions concerning the site of formation of these antibodies and the reason for their concentration around the place of injection of the vaccine. (See Cannon and Sullivan[6] for discussion.)

The results obtained with the control animals 1615 and 1627 are interesting since they offer an explanation for the observation that the repeated *intravenous* injection of a foreign protein may result ultimately in a hypersensitivity of the eyes without any *local* sensitizing injection of the antigen.[7] These two rabbits had received intravenous injections of typhoid vaccine over a period of a month and a half. The antibody content of the tissue fluids from both eyes was so high as to suggest that the intravenous injection of the antigen in these animals might produce local inflammation in these tissues.

The present experiments have demonstrated that the injection of typhoid vaccine into the anterior chamber of the rabbit's eye results in a concentration of agglutinins in certain tissues of the eye. Twelve days after the intraocular injection of the antigen the titer of agglutinins in the juices extracted from these eye tissues begins to surpass that of the juices extracted from the opposite control eye. One to 3½ months after injection the titer of agglutinins in the tissue juices of the injected eye is equal to or greater than the agglutinin titer of the serum and is on an average about 12 times as great as the agglutinin titer of the control eye tissue juice.

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† These observations agree with the findings of Madison[4] and fail to confirm those of Schamburow[5].

[4] Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1013.

[5] Schamburow, D. A., *Z. f. Hyg. u. Infektionskrank.*, 1932, **114**, 456.

[6] Cannon, P. R., and Sullivan, F. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 517.

[7] Seegal, B. C., and Seegal, D., *J. Immun.*, 1933, **25**, 221.

7161 P

An Internal Electrocardiographic Lead.*

ABRAHAM LIEBERSON AND FRANK LIBERSON. (Introduced by Nathan F. Blau.)

From the Cardiac Research Division, United States Public Health Service Hospitals, New York.

The increased interest in chest and precordial leads, which are semi-direct leads tapping the cardiac action current from points close to the anterior surface of the heart, prompted the authors to seek an equivalent lead portraying more intimately the electrical phenomena at the posterior surface and base of the heart. A lead taken from the back of the chest fails to do this, for unlike the front, where there is only ½ inch of tissue intervening between heart and electrode, there is usually 4 inches of intervening tissue posteriorly.

The difficulty was overcome by making use of the fact that the esophagus is closely attached to the posterior pericardium throughout its extent. A non-polarizable German Silver electrode, shaped somewhat like the Rehfuss tip, to which a thin flexible German Silver cable was clamped, served as an internal electrode. The cable was insulated throughout its length by rubber tubing. This electrode was swallowed in turn by 6 normal subjects, very much like the Rehfuss tube, its descent being checked by fluoroscopic examination. The esophageal electrode was connected to the right-arm side of the galvanometer, and the left-arm electrode (a flat 1 inch disc) lead to some point distant from the heart, such as the left leg or right arm of the subject.

When the internal or exploring electrode lay at the level of the auricles, the electrocardiogram demonstrated uniformly a peculiar deflection, a very sharp, steep, rapidly moving deflection, which arose from the rounded portion of the P-wave, and which was almost as tall as the QRS deflection, which it resembled. (See Fig. 1.)

The high complex described above is interpreted as the "intrinsic"

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*Approved for publication by the Surgeon General, U. S. Public Health Service.

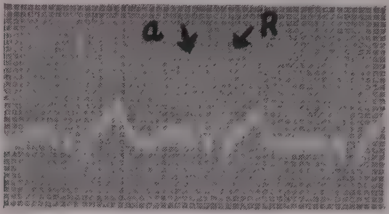

FIG. 1.

Electrocardiogram of normal male with internal electrode in lower esophagus behind the left auricle, and external electrode at a point distant from the heart, the right forearm. Note the very sharp intrinsic auricular deflection (a) which resembles the QRS complex. (R)

deflection of the underlying auricular musculature. It resembles very closely the auricular complexes that Lewis[1] obtained by leading off directly from the exposed auricle of the dog. That the deflection shows this intrinsic character is not surprising, considering that the internal electrode lies only about ¼ inch from the auricular surface, making the lead almost a direct one.

This internal lead is of great theoretical interest, since it permits study of parts of the heart, the base and the auricles, which have received very little direct study in man, and which are not readily accessible in animals, even after operation. Numerous problems related to the nature of the excitation wave in the auricles in health and disease can be studied directly by this method. The authors, for example, determined by use of the "intrinsic" auricular deflection the speed of conduction of the excitation wave in man's auricles. This study will be reported later.

We have not found any mention in the literature of the use of the *lower* esophagus as a semi-direct lead for the study of the auricles; although the *upper* esophagus and lower rectum were used as a simple *indirect* lead in dogs by Rothberger and Winterberg[2] in 1913. They were studying experimentally produced extrasystolies by the use of bi-axial leads, *i. e.,* a lead perpendicular to the conventional lead I.

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[1] Lewis, T., Meekins, J., and White, P. D., *Phil. Trans. Royal Society,* **1914,** Series B, **205,** 375.

[2] Rothberger, C. J., and Winterberg, H., *Pfluger Arch. f. d. ges. Physiol.,* **1913, 154,** 571.

7162 P

Action of Microorganisms from Soil on Type-Specific and Nontype-Specific Pneumococcus Type-I Carbohydrates.

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The report of Avery and Dubos[1] that the specific carbohydrate of pneumococcus type III was split by an enzyme from a soil microorganism appeared to offer a new approach to the study of relationships and differences between chemically and immunologically different preparations of the specific carbohydrates.

Organisms which decompose the specific carbohydrates of pneumococcus types II and III were described in a previous report.[2] We now have in pure culture a microorganism which utilizes to some extent the specific carbohydrate of pneumococcus type I. As in the study of the bacteria that decomposed the carbohydrates of pneumococcus types II and III, the precipitation test with antipneumococcus serum was used as an indication of the presence or absence of the carbohydrate, but, unlike the results obtained with these microorganisms, the precipitation reaction never entirely disappeared. It has not yet been ascertained whether this residual reaction is due to an unused portion of the original carbohydrate or to products of decomposition, which might either be present in the original sample or be formed as a result of the action of the microorganism from the soil.

In mixed culture, as it was first obtained from soil, this microorganism could not be cultivated on the purified specific carbohydrate, and, until it was isolated in pure culture, it had to be maintained on a mineral medium to which specific carbohydrate only partly purified had been added. In pure culture, the microorganism utilized the soluble specific substance obtained from broth culture and the specific carbohydrate isolated from pneumococcus type-I cells as well.

In the type-I-carbohydrate, mineral-base medium in which routine transfers are maintained, the organisms are small Gram-negative rods tapering to a point at the end. In older cultures in liquid or semisolid, saccharose, mineral medium, rods with a similar appearance are seen, together with many pleomorphic forms. No growth develops on ordinary, beef-extract, peptone agar, but small yellow colonies are obtained on mineral-base agar containing saccharose. Saccharose, 1%, and dextrose, 0.1%, in a mineral base, are utilized by the organism. Larger amounts of dextrose have a somewhat inhibitory effect on growth. Growth in a mineral base containing 1% of saccharose is vigorous.

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[1] Avery, O. T., and Dubos, Rene, *Science*, 1930, **72**, 151.

[2] Sickles, G. M., and Shaw, Myrtle, *J. Infect. Dis.*, 1933, **53**, 38.

No soluble enzyme has been obtained from this microorganism.

It is well known that the pneumococcus may become attenuated and lose, in varying degrees, its virulence, capsule formation, insusceptibility to phagocytosis, and type specificity. The antigenic activity as an immunizing agent and the production of specific carbohydrate are also altered. A nontype-specific carbohydrate has been obtained in small amounts by Wadsworth and Brown[3] from the frozen and thawed cells of such an attenuated strain, which was isolated in 1925 from the blood of a horse undergoing immunization with a typical, type-I strain and which has never regained its virulence.

Several different species of microorganisms from soil have been found to decompose this nontype-specific carbohydrate when precipitation with antipneumococcus serum is used to indicate the presence or absence of the carbohydrate. Two of the species have been investigated in more detail. One is the aerobic, spore-forming rod, described in a previous report,[2] which utilizes the specific carbohydrate of pneumococcus type III and agar. The other acts on the nontype-specific carbohydrate only and is a Gram-negative, aerobic, nonmotile, nonspore-forming rod which grows on standard media. The colonies on blood agar are small and rust-colored; on potato, the growth is a bright orange. Gelatin is liquefied, and acid is produced in dextrose, lactose, saccharose, maltose, inulin, and dextrin serum-water medium.

A soluble enzyme that decomposes the nontype-specific carbohydrate in the absence of bacterial cells has been obtained from both strains. However, no protection of mice against pneumococci of types I, II, or III has been demonstrated with these preparations.

The carbohydrate obtained from the cells of the attenuated strain, like the specific preparations from the cells of pneumococcus types I, II, and III, induces purpuric lesions when injected into mice. It was previously noted that, when the "cellular carbohydrate" from pneumococcus types II and III had been decomposed by the specific microorganism from the soil, purpura no longer appeared after injection into mice. The nontype-specific carbohydrate, after decomposition by either strain, also failed to induce purpura.

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[3] **Wadsworth, Augustus, and Brown, Rachel,** ***J. Immunol.*****, 1933, 24, 349.**

7163 P

Production of Lens Sensitivity in Rabbits by the Action of Staphylococcus Toxin.

EARL L. BURKY

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Extracts of lens[1] derived from any of the vertebrates, with the exception of rabbit lens, produce, when injected into rabbits, precipitins for all lens extracts Although a high precipitin titer can be developed neither an Arthus reaction nor an anaphylactic state can be produced in the rabbit. In a previous report[2] it was shown that Staphylococcus toxin, injected intracutaneously into rabbits, produces not only an antitoxin but also a hypersensitive state to the beef bouillon in which the toxin is produced and to beef serum. This result suggested that if such a poor antigen as beef broth could be made antigenically active, a similar antigenic effect could be developed for lens substance if this material were added to the broth. Accordingly the following experiments were undertaken.

Beef eyes, trimmed of all extraocular tissue, were dropped into boiling water for 30 seconds. With a sterile scissors a transverse corneal incision was made and the lens expressed into a tube containing 20 cc. of hormone bouillon. After a preliminary incubation period of 7 days to eliminate the contaminated tubes, the sterile tubes were inoculated with toxin-forming *Staph. aureus,* strain Ha. After 10 days' incubation at 37°C. the contents of the tubes were pooled, ½% trikresol added and filtered through a Berkefeld V filter. Injected intravenously, 0.3 cc. killed a 3000 gm. rabbit within 24 hours. This lens broth toxin precipitated with lens immune serum in dilutions as high as 1-50,000.

Rabbits were injected intracutaneously with 0.1 cc. amounts of lens broth toxin and lens extract, each in a different skin area at intervals of one week. Two rabbits were injected intravenously with

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[1] Woods, A. C., Allergy and Immunity in Ophthalmology, Johns Hopkins Press, Baltimore, 1933. Review of the literature on immunology of the lens.

[2] Burky, E. L., *J. Immunol.*, 1933, **25**, 419.

5 cc. amounts of lens extracts once a week. Other details of technique have been outlined elsewhere.[1, 2]

Beef, swine and rabbit lens extracts produced areas of swelling, erythema and occasional necrosis greater than 2x2 cm. in 8 of 9 rabbits after the 3rd or 4th injection of lens broth toxin. The ninth rabbit did not become sensitive to lens until after the 6th injection. Preceding this experiment 4 rabbits were repeatedly injected intracutaneously with mixtures of beef serum and lens extract without developing sensitivity or precipitins for lens extract although a marked Arthus reaction to beef serum developed. The intravenously injected rabbits were not skin reactive after the 5th intravenous injection of lens extract.

TABLE I.

No. Rabbits	No. Injections	Material injected	Skin Reactions to Lens Extract	Precipitin titers Dilution of lens	Intraocular Reactions
8	3	Lens toxin, .1 cc. Lens extract, 1 cc. intracutaneous	Positive after 3rd or 4th injection	1-50,000 or more	Positive See text
1	6	Lens toxin, .1 cc. Lens extract, .1 cc. intracutaneous	Negative after 4th injection	1-5000	Negative
2	5	Lens extract, 5 cc. intravenous	Negative	1-5000	”
4		Normal controls	Not done	Negative	”
4	8	Beef serum + lens extract, 0.4 cc. intracutaneous	Negative	”	”

The 9 rabbits in the intracutaneous group and the 2 rabbits in the intravenous group developed precipitins for lens extract. The highest titers were found in the sensitive animals (1-50,000 or more). The intravenously injected animals after 5 injections had titers not higher than 1-5,000. Lens precipitins were not present in these serums before immunization was begun.

To determine whether these skin reactions had any real significance the 11 rabbits and 4 normal controls were treated in the following manner after the 4th intracutaneous injection. Under cocaine anesthesia, a 25 G. needle attached to a syringe was inserted into the anterior chamber and about 0.2 cc. of aqueous humor was drawn into the syringe and then injected into the lens.

The sensitive animals developed marked intraocular inflammation characterized by conjunctival and pericorneal injection, steamy cornea with vascularization, positive aqueous ray and plastic iritis. The ocular inflammation continued for at least 7 days in the eyes not removed for histological study before this time. The immune but insensitive animals reacted like the normal controls with a purely traumatic reaction that subsided in less than 7 days.

Summary. Rabbits cannot be sensitized to lens protein by ordinary methods. By the intermediary action of Staphylococcus toxin a skin and ocular sensitivity to lens substance has been developed. Precipitin titers, higher than those developed by ordinary methods, are also produced. This method, to our knowledge, has not been previously reported and may be of wider application in the experimental study of hypersensitive states.

A more complete report will be published in an ophthalmological journal.

7164 C

Phosphatase Activity of the Bones and Kidneys in Thyrotoxicosis.*

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From the Laboratories of the Collis P. Huntington Memorial Hospital, Harvard University.

Considerable experimental work has been done within the past few years on the phosphatase activity of various bodily tissues. Robison[1] showed that soluble salts of hexosemonophosphoric esters were hydrolyzed by an enzyme present in ossifying cartilage, kidney, and the shafts of long bones. Kay,[2] Robison,[1] and others have shown that the intestinal mucosa, kidney, and whole bone contain the greatest concentration of this enzyme. Furthermore, the plasma and tissue phosphatase activity has been shown to vary from the normal in certain pathological conditions. Page[3] has shown that bone phosphatase is diminished if an excess of vitamin D is fed to an animal, along with the coincident decalcification of bone and metastatic calcification of other tissues. Parathormone has also been shown[4] to depress the activity of the bone enzyme in rats, though not to the same extent as the administration of vitamin D.

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* This is reprint No. 324 of the Cancer Commission of Harvard University.

[1] Robison, R., *Biochem. J.*, 1923, **17**, 286.

[2] Kay, H. D., *Biochem. J.*, 1928, **22**, 855.

[3] Baumgartner, L., King, E. J., and Page, I. H., *Biochem. Z.*, 1929, **213**, 170.

[4] Page, I. H., and Reside, M., *Biochem. Z.*, 1930, **223**, 171; 1930, **226**, 273. Page, I. H., *Biochem. Z.*, 1930, **223**, 222.

It is known that the thyroid gland also profoundly influences calcium and phosphorus metabolism. In exophthalmic goiter the excretion of calcium and phosphorus is markedly increased and there is decalcification of bone though the level of calcium and phosphorus in the blood remains normal.[5] Kay[6] and Hunter[7] have demonstrated an increase in the phosphatase activity of the plasma in hyperthyroidism but a correlation of the increased excretion of calcium and phosphorus and the decalcification of bone with the phosphatase activity of the kidneys and bones, respectively, has not been attempted. The present study was undertaken to determine whether such a correlation exists by measuring the phosphatase content of the bones and kidneys of animals rendered thyrotoxic by injections of thyroxin.

In numerous preliminary experiments, the results coincided in general with those here reported. Two litters of pure strain, white rats were chosen, 50 and 100 days old, respectively, at the onset of the

TABLE I

No.	Sex	Wt. when killed, gm.	Wt. at start, gm.	Medication	Wt. of kidneys, gm.	Wt. of bones gm.	Kidney Phosphatase Units/gm.	Bone Phosphatase Units/gm.
Litter No. 1—50 days old.								
1	M.	78.0	74.0	0.2 mg. thyroxin daily for 10 days	.92	.71	11.7	13.1
2	M.	84.5	83.8	”	.97	.83	13.1	13.3
3	M.	79.5	80.0	”	.83	.71	13.9	16.9
4	M.	64.8	74.7	”	.74	.71	16.2	21.1
5	F.	69.4	71.3	No medication	.64	.73	17.9	15.2
6	M.	75.0	78.4	”	.72	.77	18.8	21.3
7	M.	81.0	73.8	”	.81	.76	11.8	14.2
8	F.	83.4	73.0	”	.74	.77	15.1	14.2
Litter No. 2—100 days old								
1	M.	118.8	125.0	0.4 mg. thyroxin daily for 18 days	1.30	1.09	9.2	16.8
2	F.	111.0	101.0	”	1.29	0.92	7.4	23.0
3	M.	102.0	122.0	”	1.33	0.88	4.7	23.1
4	F.	100.0	98.0	”	1.16	0.86	11.3	17.1
5	M.	106.0	111.5	”	1.19	0.88	9.7	14.9
6	M.	146.5	115.5	No medication	0.96	1.02	13.9	26.7
7	M.	174.6	124.5	”	1.18	0.88	9.7	14.9
8	F.	122.0	97.0	”	0.89	0.87	21.8	18.8
9	F.	112.0	97.0	”	0.77	0.85	35.2	27.0

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[5] Aub, J. C., Bauer, W., Heath, C., and Ropes, M., *J. Clin. Invest.*, 1929, **7**, 97

[6] Kay, H. D., *Phys. Rev.*, 1932, **12**, 384.

[7] Hunter, D., *Quart. J. Med.*, 1930, **24**, 393.

experiment. There were 17 animals in all (Table I). Approximately one-half of each litter received injections of thyroxin, while their litter mates, kept in the same environmental conditions, were used as controls. The rats in the first litter received 0.2 mg. of thyroxin intramuscularly daily for 10 days, being killed on the eleventh and twelfth days. Those in the second litter were injected with 0.4 mg. of thyroxin per day for 18 days and were killed on the nineteenth and twentieth days. The injections were all given in the thigh muscles, alternate legs being used for successive injections. The thyroxin used was the Squibb crystalline product and was freshly dissolved every few days in N/50 sodium hydroxide in concentration such that 0.1 cc. was used for each injection. The effects of the thyroxin were followed by observing the loss of weight, or failure to gain weight, of the injected animals as compared with the controls.

In the litter receiving 0.2 mg. of thyroxin for 10 days, the injected animals showed an average loss of weight of 1.4 gm. while the controls showed an average gain of 3.4 gm. In the litter which received the heavier dose of 0.4 mg. for 18 days, the treated animals lost an average of 4.9 gm., whereas the untreated controls gained an average of 23.3 gm. The animals were given the same daily rations and at the end of the experiment the treated animals had eaten all of their food while 92 gm. of food were still unconsumed in one of the cages containing controls.

The method of determining the phosphatase content of the bones and kidneys was taken from Kay[8] with certain modifications. One unit is the amount of enzyme necessary to split one milligram of inorganic phosphorus from an excess of substrate during an incubation period of 2 hours at 37°C. and a pH of 8.8. Four bones, the femora and humeri, were removed from each animal and the soft tissue down to the periosteum removed. The 2 kidneys were removed and decapsulated. The bones and kidneys were thoroughly crushed in separate flasks and weighed. Twenty parts of water were added to each flask and the flasks agitated at room temperature for 48 hours.

The extract was then centrifuged for 6 minutes at 800 revolutions per minute, the same in each case, and 0.5 cc. of the supernatant fluid was put in 5 cc. of a combined reagent and incubated for 2 hours at 37°C. Control tubes containing 0.5 cc. of the extract, some in water and others in combined reagent, were set up at the same time. The control tubes were immediately inactivated by the addition of 2 cc. of trichloracetic acid. At the end of the incubation period, trichloracetic acid was also added to the incubated tubes, the mixtures all filtered, and the inorganic phosphorus present determined by the method of Fiske and Subbarow.[9]

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[8] **Kay, H. D.,** ***J. Biol. Chem.*****, 1930, 89, 235.**

The phosphatase content of the kidneys was found to vary markedly in all the animals tested. The kidneys of the thyrotoxic animals contained from 4.7 to 16.1 units of phosphatase per gram of tissue, averaging 10.8 units. In their respective controls the phosphatase content varied from 11.8 to 35.2 units per gram of tissue with an average of 19.2 units. This suggests that thyroid reduces the phosphatase content of kidney somewhat but the significance of this is diminished by the wide variations in results. An incidental finding, although the series is small, is the absolute and relative increase in weight of the kidneys in the thyrotoxic animals.

The bones showed less variation. In the thyrotoxic animals, the phosphatase content varied from 13.1 to 23.1 units, with an average of 17.7 units; whereas their controls showed a variation from 14.2 to 27.0 units with an average of 19.6 units of phosphatase per gram of tissue. Obviously, there is no significant variation from the controls produced by the injection of large doses of thyroxin.

Summary and Conclusions. The phosphatase activity of the bones and kidneys of thyrotoxic white rats has been studied. The kidneys of the thyrotoxic animals were found to be larger than those of their controls and the phosphatase content was lower per gram of tissue on the average, but there was such a wide range of variation that the results were not conclusive in so small a series. The phosphatase content of the bones of the thyrotoxic animals was the same as that of their controls. The decalcification in thyrotoxicosis is thus probably not accompanied by a change in the phosphatase activity of the bones.

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[9] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1923, **66**, 375.

7165 P

Acute Barbital Poisoning in Dehydration and Diuresis.

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In several previous communications[1, 2, 3] we brought forth some evidence showing that diuretic measures increased neither the rate nor the percentage of elimination of barbital, nor did they shorten the recovery period from barbital depression. Since Gower and Van de Erve[4] recently again stated definitely that diuresis hastens recovery from barbital poisoning, and because of the practical therapeutic importance of the matter involved we decided to reinvestigate the problem.

Dogs were used throughout the experiments. Sodium barbital was administered in doses ranging from 225 to 600 mg. per kg. intravenously. Diuresis was produced by intravenous injection of appropriate doses of 10% glucose, physiological salt solution, 3% calcium chloride, and from 0.5 to 1.0% ammonium chloride. In

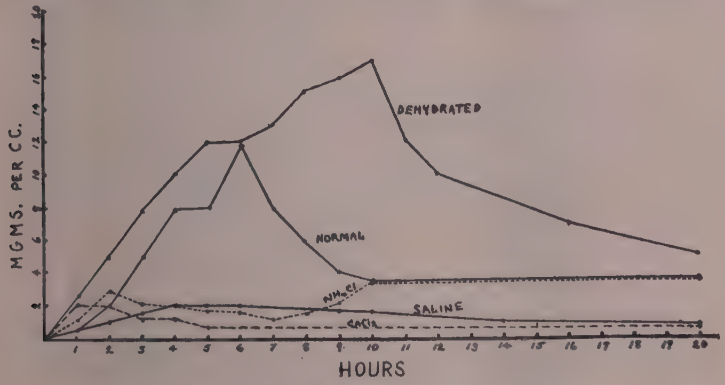

Figure 1.

Concentration of barbital in milligrams per cubic centimeter of urine over a period of 20 hours. The normal, dehydrated, and the saline treated dog received 300 mg. per kilo, the ammonium chloride, and the calcium chloride treated dogs received 225 mg. of sodium barbital per kilo.

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[1] Koppanyi, Murphy, and Krop, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 542.

[2] Koppanyi, Murphy, and Krop, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1495.

[3] Koppanyi, Murphy, and Krop, *Arch. Int. Pharm. Therap.*, 1933, **46**, 76.

[4] Gower and Van de Erve, *J. Pharm. and Exp. Therap.*, 1933, **48**, 141.

contrast to the diuretic measures, dogs were dehydrated by withholding of fluids for 48 hours and by bleeding (12 cc. of blood per kg. withdrawn from the saphenous vein) immediately before barbital administration.

These intravenous injections were carried out by slow infusion with a burette, the total volume being injected during a period of from 10 to 12 hours. The animals, if they survived either procedure, were again given the same dose of barbital about 2 weeks following the first injection.

TABLE I.

Dog No.	Dose Na Barbital mg.	Treatment	Urine volume during 48 hours cc.	excreted %	Recovery in hours	Remarks
1	300	Dehydration and 12 cc. per kg. bleeding	255	30.1	Died in 48	Glomerular damage to the kidneys
2	300	Same	370	84.0	43½	
3	300	Same	115	82.0	40	
4	300	1000 cc. Ringer, 100 cc. per hr.	1036	75.5	47	
5	300	Same	829	81.0	46½	
6	300	No treatment	439	94.5	26	
7	300	”	114	62.9	48	
8	300	”	347	75.7	46	
9	300	”			46	
10	300	”	436	75.8	45	
11	300	”			25	
12	226	720 cc. 0.5% NH_4Cl in 8 hrs.	579	85.5	28	
13	225	236 cc. 5% NH_4Cl by mouth	665	82.1	22½	
14	225	180 cc. 3% $CaCl_2$ in 5 hrs.	165	3.6	Died in 11 hrs.	
15	225	160 cc. 3% $CaCl_2$ in 6 hrs.	446	23.8	Accidentally killed in 6½ hrs.	
16	225	720 cc. 0.5% NH_4Cl in 8 hrs.	615	66.4	47	Sick dog
17	600	Same	469	29.1	Died in 7 hrs.	
18	600	300 cc. 1% NH_4Cl in 3 hrs.	35	8.6	” ” 3 hrs.	
19	600	No treatment	66	1.9	” ” 2 hrs.	
20	600	179 cc. 2.5% Na_2SO_4 in 30 min.	48	2.3	” ” 1 hr.	
21	600	10 mg. picrotoxin every 20 min. for 100 min. plus 20 cc. .5% NH_4Cl. 160 cc. .5% NH_4Cl by vein in 6½ hrs. 275 cc. .5% NH_4Cl by muscle in 2½ hrs. 8 mg. picrotoxin after 5 hrs.	299	59.3	” ” 9½ hrs.	Aver. conc. barbital in urine—9 mg. per cc.

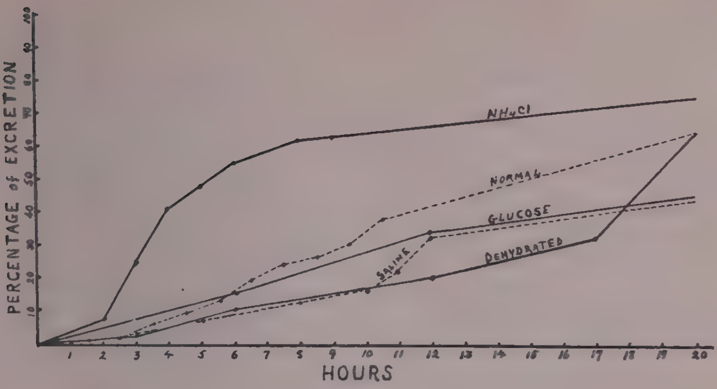

FIGURE 2.

Percentage of excretion of barbital in urine of a dehydrated, normal, and 3 diuretic dogs (10% glucose, physiological salt solution, and ammonium chloride.)

As Table I shows, dehydration did not prolong the recovery time nor did the saline diuresis carried out according to the method of Gower and Van de Erve shorten the duration of the barbital narcosis. In fact, no diuretic procedure seemed to have any significant effect on the duration of barbital anesthesia. However, as the comparison of Graphs I and II shows, there is a very striking effect of NH_4Cl on the rate of elimination of barbital.

In conclusion we might say that the supposed beneficial effects of glucose and physiologic saline in barbital poisoning may be disregarded and that ammonium chloride diuresis even though it did not appreciably shorten the period of depression did definitely increase, unlike glucose or salt solution, the rate of elimination of barbital.

7166

Failure to Enhance the Growth of B. Leprae by Wherry's Gaseous Method (CO_2, O_2 Environment).

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One of us (Duval[1]) reported the observation that the acid-fast bacillus of Hansen in removed pieces of leprous tissue continued slowly, over a period of months, to multiply and that the microscopic colony-like masses, known as "globi", attained many times their original size. Duval further observed that the proliferation of *B. leprae*, regardless of any special atmospheric environment, progressed *pari passu* with the autolysis taking place in the transplanted leprous tissue and that growth continued as long as the end-products of protein digestion were available. Continued growth in subsequent transplants occurred only in a medium containing certain protein-split products (amino-acids).

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[1] Duval, C. W., *J. Exp. Med.*, 1910, **12**, 649.

The recent claims of Wherry[2] and those of Soule and McKinley[3] for the CO_2, O_2 method of culturing *B. leprae* led us to employ this method in our recent studies of the *in vitro* cultural requirements of the Hansen bacillus of human leprosy. Wherry notes that it is essential to furnish suitable nutritives as well as CO_2, O_2 environment for the proliferation *in vitro* of *B. leprae*. In contrast, Soule and McKinley emphasize the necessity of this atmospheric condition to such an extent that the nutritive requirement is lost and becomes a negligible factor.

The leprous tissue used in the cultivation experiments were obtained through the kindness of Dr. Denney of Carville, La. It consisted of the subcutaneous leprous nodules and other material known as the leprous "abscess" which were extremely rich in acid-fast bacilli. Some of this material was free from contamination as evidenced by microscopic and cultural examinations, while others were contaminated with some undetermined gram-positive micrococcus. All tissues were cut aseptically into small pieces or emulsed in a mortar. The contaminated material was further treated with a 3% NaOH for one hour and then washed several times in Ringer's solution.

A large series of cultures were prepared upon a variety of solid and liquid media, containing chick embryo tissue, placenta autolysate, banana extract and certain purified amino-acids in various proportions. The inoculated media were placed in Novy jars and maintained for weeks at 37°C. under a gaseous tension of 10% CO_2 and 40% O_2. Duplicate cultures were subjected to ordinary atmospheric environment, media and temperature.

Careful examination of the cultures after periods of 4 and 6 weeks failed to show that the special gaseous environment affected in any way the proliferation of *B. leprae*. The CO_2 and O_2 atmosphere neither inhibited nor enhanced the *in vitro* multiplication. Our negative results with the Wherry method are in entire accord with those of Oliver *et al.*[4] and the British bacteriologists[5] in India who likewise have found the method of no value in the cultivation of the Hansen bacillus.

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[2] Wherry, *J. Infec. Dis.*, 1930, **46**, 263.

[3] Soule and McKinley, *Am. J. Trop. Med.*, 1932, **12**, 1.

The fact that *B. leprae* may continue to proliferate in leprous tissue that has been removed from the human host regardless of any specially supplied cultural conditions, possibly explains the so-called "positive culture" and "micro-colonies" claimed by Soule and McKinley. Furthermore, they state that *B. leprae* does not grow with greater ease throughout successive transplants, but with increasing difficulty. This indicates to us that proliferation became less and less and ultimately ceased, because the transferred culture inoculum no longer contained any of the original tissue products of protein digestion which Clegg,[6] Duval,[7] and Kedrowski[8] have shown is necessary to sustain the primary *in vitro* growth of *B. leprae*. Soule and McKinley have failed apparently to appreciate the importance of the amino-acid nutritives in the initial recovery and maintenance of successive generations of *B. leprae* that are removed from the influence of the living host.

7167 C

Pathologic Changes in the Organs of Scorbutic Guinea Pigs.

OTTO A. BESSEY, MAUD L. MENTEN AND C. G. KING

From the Departments of Pathology, University of Pittsburgh and Children's Hospital; and Department of Chemistry, University of Pittsburgh.*

Recently Waugh and King[1] have shown that crystalline vitamin C isolated from lemon juice is identical with the hexuronic acid previously prepared from beef adrenals. With the more exact knowledge of the chemical nature of vitamin C it was deemed desirable to restudy some of the scorbutic lesions produced in organs. The most conspicuous gross lesions occur in the joints, bones and teeth and the pathologic appearance of these have been reported in detail by Holst and Frölich,[2] Hess,[3] Höjer and Westin,[4] Meyer and McCormick,[5] and others. Changes in the organs have received less attention.

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[4] Oliver, *Philippine J. Sc.*, 1931, **46**, 611.

[5] Leprosy in India, *Editorial Review*, Vol. 5, No. 4, October, 1933.

[6] Clegg, *Philippine J. Sci.*, 1909, **4**, 403.

[7] Duval, C. W., *J. Exp. Med.*, 1911, **13**, 365.

[8] Kedrowski, *Z. F. Hyg. u Infektionkrankheiten*, 1910, **66**, 1.

*Contribution No. 267 from the Department of Chemistry.

[1] Waugh, W. and King, C. G., *Science*, 1932, **75**, 357; *J. Biol. Chem.*, 1932, **97**, 325.

Guinea pigs were fed on the basal vitamin C free diet of Sherman,[6] supplemented by cod liver oil and yeast to supply adequate quantities of vitamins A, B, D, and G. After a preliminary survey of the histopathology of the organs and tissues, work was concentrated on adrenals, heart, testes and liver in which evidence of scorbutic effects was most marked. The pathologic bone changes studied by us did not differ from those reported by other investigators.

Microchemical preparations of formalin fixed and fresh tissue, both teased and cut with freezing microtome, were compared after treatment with Sudan iv, osmic acid, Nile blue sulphate and Nile pink[7] for distribution of fat. Since practically identical pictures were obtained with all of these reagents, in later work Sudan iv and formalin fixed tissue was chosen. Hematoxylin and eosin stained sections were used for controls.

The distribution of cholesterol esters was indicated by the presence of doubly refractive materials in warmed formalin sections when examined with Nicol's prisms. The distribution and relative concentration of vitamin C in the tissues, particularly in the adrenals, was followed by the intensity of the reduction of 0.4% neutral silver nitrate.

The cortex of normal adrenals was invariably heavily laden with fat-staining material but this was not uniformly distributed. The zona fasciculata seldom showed any appreciable amount, the zona reticularis contained variable, but usually small amounts, and the zona fasciculata was loaded with lipids. The lipid was present only in the cytoplasm where it appeared to be located as a film on the periphery of fairly large granules. The nucleus was free from demonstrable fat. When considerable amounts of the fat had become free from the granules, it collected in droplets, which frequently fused to form long lines bordering the vascular channels. The unsaturated nature of these fatty materials was shown by the rapidity with which they reacted with osmic acid. The doubly refractive cholesterol esters were uniformly distributed in the zona fasciculata, where they were at a maximum in the normal adrenal. These substances were not apparent in the remainder of the cortex. A high content of fat is maintained in the adrenal until a fairly severe scurvy develops. In guinea pigs deprived of vitamin C, little or no apparent reduction appeared in the fat content until several days before death. At this time the fat in the middle third of the zona fasciculata stained less intensely due to the fatty covering of the granules having been transferred into the intervening thinner cytoplasm, whence it passed in droplets to the vessels, which transported it towards the medulla. Occasionally capillaries loaded with this freed fat and stained with osmic acid, appeared as thin black lines lying at right angles to the capsule of the gland. This depletion of fat spread towards the periphery of the fasciculata, and, if the animal showed a prolonged survival, the fat entirely disappeared from the outer two-thirds of this zone. The inner third retained an apparently normal fat content. In a few animals a thin rim of fat remained in that part of the fasciculata contiguous to the zona glomerulosa. Immediately beneath this was a fat free band comprising about one-fourth of the fasciculata with the remaining inner half of the fasciculata showing a fairly normal amount of fat. The zona glomerulosa at this stage stained more deeply. The zona reticularis likewise showed a deeper color than normal. Coterminous with the decrease of demonstrable fat in the fasciculata there occurred a comparable diminution in doubly refractive material. These observations agree in detail with those reported by Iwabuchi.[8] In guinea pigs, where the scurvy developed rapidly, patchy and irregular fat depletion separated by intervening fat stained areas was observed. Such a distribution of fat always occurred in association with hemorrhagic foci. In such animals the transport of the freed fat towards and in the vessels was more or less limited to those hemorrhagic foci and at death the picture of a uniformly depleted band comprising the outer half or two-thirds of the fasciculata was lacking. When deviation from the first fat picture described occurred, undoubtedly contributory factors, *e. g.,* infection, were involved in the rapid withdrawal of vitamin C from the adrenals. The medulla of adrenals, with fat depleted cortices, showed little or no chromaffin pigment which is consistent with the decrease of adrenalin found by McCarrison[9] and Ohata.[10]

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[2] Holst, A., and Frölich, T., *Z. f. Hyg. u. Infektionsk.*, 1912, **72**, 1.

[3] Hess, A. F., *Scurvy, Past and Present*, Lippincott, 1921, Phila.

[4] Hojer, J., and Westin G., *Dental Cosmos*, 1925, **57**, 1.

[5] Meyer, A. W., and McCormick, L. M., *Studies on Scurvy*, Stanford Univ. Press, 1928.

[6] Sherman, H. C., La Mer, V. R., and Campbell, H. L., *J. Am. Chem. Soc.*, 1922, **44**, 165.

[7] Rettie, T., *J. Path. and Bact.*, 1931, **34**, 595.

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[8] Iwabuchi, T., *Beitrage z. path. Anat. u. z. Allgem. Path.,* 1922, **19**, 440.

[9] McCarrison, R., *Brit. Med. J.,* 1919, **2**, 200.

[10] Ohata, S., *J. Biochem.* (Tokyo), 1930, **12**, 419

Parallel to the disappearance of cortical fat staining material the doubly refractive cholesterol also disappeared. This bears out the chemical findings reported by Nagayama and Tagaya[11] on the decrease in cholesterol in the adrenal cortex in scurvy. The amount of doubly refractive material was directly proportional to the amount of demonstrable fat in the fasciculata. These various lipid pictures we have found to appear consistently in several dozen guinea pigs. Inanition *per se* did not produce a comparable change in lipid distribution. Comparison of hematoxylin sections with the microchemical preparations showed swelling, pyknosis and lysis of cells. In animals dying acutely, cortical hemorrhage was common. The intimate relationship of vitamin C to fat content of adrenal was shown by a series of studies with 0.4% neutral silver nitrate. Indeed, this technic offers a rough assay of the vitamin C content of tissue in agreement with the findings in these laboratories.

Marked differences were observed in the staining of the cut surface of the adrenals in normal and scorbutic pigs similar to that reported by Moore and Ray,[12] Miller, Siehrs and Brazda[13] and Hamilton.[14] Immersion of the cut surface of the adrenal of a normal guinea pig, one fed on a liberal diet of spinach, or injected with the pure vitamin C, in 0.4% of neutral silver nitrate, produced a rapid and intense blackening. This blackening developed in a few minutes in the light but required about 30 minutes in the dark. In animals deprived of vitamin C the intensity of this silver deposit decreased gradually until the sixth day, when it appeared to reach a minimum which was maintained until the death of the animal from scurvy (16-48 days). The adrenals from pigs which had received no vitamin C for 10 days and were then fed liberal quantities of spinach showed intense staining 3 hours subsequent to the feeding. A similar effect was noticeable in a shorter time after an intramuscular injection of pure vitamin C. The adrenals from pigs fed on a vitamin C level, such that at the end of a 60 day period showed distinct scorbutic lesions, stained like those glands from pigs on a 5-day vitamin C free diet.

The black silver deposit begins to appear first in the zona fasciculata and then the reduction gradually spreads to include the zona reticularis. The change in the cells during freezing and cutting and the subsequent film deposit of silver on the cut surface presents difficulty in determining the exact location at which the reduction appears. The most intensely stained areas are in the small channels emptying into the blood vessels of the zona fasciculata. The reducing property of the adrenal cortex is undoubtedly intimately associated with the presence of both vitamin C and lipids. An attempt to use the silver nitrate solutions suggested by Macallum to demonstrate the distribution of chlorides and phosphates was unsuccessful, because of the rapid reduction of silver nitrate by the vitamin. The demonstration of potassium by the Macallum reagent was likewise unsuccessful in the adrenal. Although quantities of potassium were present in the normal as well as the scorbutic adrenal so much was freed during the freezing and manipulation of the tissue that a thin film formed on the surface of the section and obscured all cellular detail.

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[11] Nagayama, T., and Tagaya, T., *J. Biochem.* (Tokyo), 1929, **11**, 225.

[12] Moore, T., and Ray, S. N., *Nature,* 1932, **130**, 997.

[13] Miller, C. O., Siehrs, A. E., Brazda, F. G., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 636, 696.

[14] Hamilton, B. H., Jr., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1355.

Coincident with the extreme lipid alteration in the adrenal extensive fatty degeneration occurred in foci irregularly distributed in the myocardium. This began by a shifting of the fat from the dark or anisotropic band into the light band where it progressively increased until droplets were formed. These fused with loss of morphology of the myocardial cell. This apparently constituted an irreversible stage and formed the beginning of the typical patches of fatty degeneration similar to those seen in diphtheria. Fatty changes in skeletal muscle were similar in pathogenesis to those formed in the myocardium.

Fatty infiltration of the liver was found in many of the animals with varying degrees of scurvy and was so marked in the terminal stages that the cells of the entire lobule were almost entirely composed of fat. This infiltration was associated with fatty degeneration which probably formed the primary lesion. Marked congestion of the liver was frequently encountered and in a few animals irregularly distributed hemorrhages were present.

In severe scurvy, extreme degenerative changes, with death of germinal epithelium and spermatozoa, were noted in the testis. The heads of the spermatozoa sometimes persisted as homogeneous dark staining masses in deeply eosin stained cytoplasm. Persistence of fairly well stained cells of Sertoli, among degenerating epithelium, as reported by Medes[15] was found only in the early and milder stages of scurvy. Another occasional observation was the swollen edematous appearance of the connective tissue in animals dying with extreme manifestations of scurvy. This was well exemplified in the periportal connective tissue, splenic septa, as well as in the media of small arterioles of the lungs. In the lung such vessels occasionally presented the appearance of obliterative endarteritis. While it is impossible to exclude such contributory factors as may be supplied by spontaneous infections in the development of these vascular changes we feel such changes are to be interpreted primarily as alterations in the colloidal state of the collagen due to lack of vitamin C as postulated by Wolbach and Howe.[16] The other lesions noted were less constant. Patches of degenerating intestinal mucosa formed the basis of the ulcerated areas seen in the gross, mostly in the small gut. In the spleen no characteristic scorbutic changes were observed unless one considers the brown hemolyzed intra and extra cellular pigment as such. The presence of occasional hydropic degeneration of the islets of Langerhans was probably only an indication of spontaneous intestinal infection.[17]

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[15] **Medes, G., and Lindsay, B., *Am. J. Anat.*, 1926, 37, 213.**

Summary. The most characteristic lesion found in the organs of scorbutic guinea pigs was the depletion of fat and cholesterol from the cortex of the adrenal. Fatty degeneration of the myocardium, marked degeneration of testicular germinal epithelium and spermatozoa and swelling of connective tissue were also observed in extreme scurvy.

7168 P

Chemical Determination of Pregnancy.

J. PAUL VISSCHER AND DONALD E. BOWMAN.

From the Biological Laboratory, Western Reserve University.

We have found that a fair degree of accuracy can be obtained in testing for pregnancy by oxidizing 1 cc. of urine with 1 drop of 0.5% hydrogen peroxide, after which 5 drops of a 1% aqueous solution of phenylhydrazine hydrochloride are added and 5 drops of a 5% aqueous solution of methyl cyanide, followed by 5 drops of concentrated hydrochloric acid. It is then put into a boiling water bath and allowed to react for 25 minutes. After this time the reaction is said to be positive if a russet color develops and a flocculent precipitate appears. A negative reaction is shown by the absence of a precipitate or the presence of a powdery one and usually a straw color. All reagents should be fresh at the time the tests are made.

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[16] Wolbach, S. B., and Howe, P. R., *Arch. Path.*, 1926, **1**, 1.

[17] Thomas, B. G. H., *J. Infect. Dis.*, 1924, **35**, 407.

It was found that a modification of this test, which appears to be more sensitive for certain of the hormones, could be adapted to titration methods. This modification is carried out as follows:

To 1 cc. of urine, one drop of 0.5% hydrogen peroxide is added and this allowed to stand for 3 minutes. Five drops of a 1% solution of phenylhydrazine hydrochloride are added, followed by 5 drops of a 5% aqueous solution of potassium ferricyanide. This is allowed to stand for 10 minutes at room temperature and then put in a boiling water bath for 15 minutes. Upon removal from the water bath, one drop of concentrated hydrochloric acid is added and this is followed by an excess of sodium hydroxide. Dilute hydrochloric acid is then added by titration until a definite end point is obtained. (The color changes from an orange to a green to a prussian blue.) It was found that it required much less dilute hydrochloric acid to obtain the end point in pregnancy specimens than was required in the case of non-pregnancy female urine specimens, and that various pregnancy tests fall in a given range, while normal tests fall in another more restricted range.

Total number of established cases tested	317
Number correct	295
% of accuracy (approx.)	93

In addition to the cases of normal pregnancy, we have had opportunity to study a limited number of irregular cases with significant results.

At the time of the writing of this report, 40 tests have been made on portions of specimens used for the Aschheim-Zondek test for pregnancy. Of this number, 33 gave the same results in both tests. Three gave a very faint positive test by the chemical method, but negative by the Aschheim-Zondek; one gave a negative test by our method and a positive reaction with a rabbit, but was found by operation to be a uterine fibroid growth. The remaining 3 tests did not agree but have not yet been determined clinically. Three doubtful cases were tested and compared with results of the Friedman test. Agreement was obtained in all.

7169 P

Early Behavior of the Embryos of Carrier-Pigeons.

HIDEOMI TUGE. (Introduced by G. E. Coghill.)

*From the Biological Institute, Tohoku Imperial University, Sendai.**

Active movements, as opposed to the passive movements due to heart-beat, begin in embryos of the pigeon of 105 ± 5 hours' incubation. These earliest movements, weak and infrequent, are extensions and flexions of the head. By 115 ± 5 hours these movements have come to involve the neck, trunk, rump and tail in the order mentioned. All these movements are spontaneous, and the wings and legs move only in perfect integration with the trunk. These total movements increase in frequency and amplitude, and eventually several parts of the axial musculature move discretely. Even at this stage the embryos do not respond to chemical or tactile stimulation, except possibly in rare cases in the latter part of the period.

The first reactions to tactile stimuli are in response to stimulation on the snout at 125 ± 5 hours, although in rare cases sensitivity to touch on the upper part of the neck may appear at about the same time. The response is at first flexion of the head and neck, and sometimes the trunk. After several hours the reaction comes to involve the tail and rump, and then the wings and legs, which, under these circumstances also, move only in complete integration with the trunk. Meanwhile the reflexogenous zone extends to the shoulder, trunk and rump in cephalo-caudal progression. In embryos of 135 ± 5 hours the tail moves with the rump, sometimes also with the leg, in response to touch on the base of the tail; and a little later the wing and the leg respond to stimulation on the shoulder or lower part of the neck, but embryos of this age do not respond to stimulation on the wing or leg. Embryos of 155 ± 10 hours exhibit local reflexes of the wings, legs and tail in response to stimulation on the respective appendages.

The development of reflexes of the eyelid, eyeball, digits, mandibles and anus, together with hatching, will be described in the final paper.

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* The study was carried out at the Japanese Military Carrier-Pigeons Corp. in Tokyo, where nearly 2000 pigeons are kept.

7170 P

Vitamin B Complex and Gastric Secretion.

D. R. WEBSTER AND J. C. ARMOUR. (Introduced by B. P. Babkin.)

From the Department of Physiology, McGill University, Montreal.

Webster and Armour[1] reported that artificially fed dogs with oesophagotomy, gastric fistula, obstructed pylorus and duodenal fistula, in which a condition of practically total avitaminosis had been induced, developed a complete achlorhydria without anorexia and inanition. Administration of powdered brewer's yeast restored the normal secretory activity of the stomach in 3 days. This indicated that for the normal activity of the gastric glands it is necessary that the vitamins contained in yeast, presumably vitamin B complex, be present in the body.

Two experiments on a similarly operated animal are here reported. The aim was to exclude as causative of achlorhydria other vitamins than vitamins B, and to determine if possible which of the latter is essential for the normal functioning of the gastric mucosa.

First Experiment. The dog, kept on Scott and Ivy's[2] food mixture, was in excellent condition 46 days after the pylorus had been obstructed. This diet was replaced by a diet of glucose, casein, sodium chloride and olive oil. As in the previous experiments the secretion of gastric juice in response to food introduced into the duodenum, subcutaneous administration of 1 mg. histamine and sham feeding gradually diminished. On the 17th day of experiment 50 cc. of olive oil in the diet were replaced by 50 cc. of cod liver oil in order to supply the body with vitamins A and D. In spite of this change in the diet, 2 days later 1 mg. of histamine provoked only 3.0 cc. of mucoid secretion as compared with 40.5 cc. under normal conditions, and the next day only 2.6 cc. On the 21st day daily administration of 10 gm. of brewer's powdered yeast was begun and the special diet was replaced by Scott and Ivy's food mixture. Four days later 1 mg. of histamine provoked an enormous secretion (93.0 cc.) of regular gastric juice. Two days after this, 4 minutes' sham feeding gave 31.5 cc. in 40 minutes. During the whole experimental period the animal lost only 1.6 kg. This experiment shows that the presence of vitamin B complex in the body is absolutely essential for the activity of the gastric glands.

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[1] Webster, D. R., and Armour, J. C., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1297.

[2] Scott, H. G., and Ivy, A. C., *Ann. Surg.*, 1931, **93**, 1197.

Second Experiment. Kept on the usual food mixture for 14 days, the dog regained its former weight (16 kg.) and was put on a diet of glucose, casein, sodium chloride and cod liver oil. At the beginning of the third week the total volume of gastric secretion in response to duodenal feeding markedly diminished. On the 19th day histamine (1 mg.) produced only 11.0 cc. of gastric secretion. On and after that day 10 to 30 gm. of yeast, autoclaved for 1¼ hours at 122°C. under 16 lb. pressure, were added to the daily diet, which otherwise was not changed. In spite of this the total secretion for the first few days was still very low (on the 6th day 1 mg. of histamine gave only 1.7 cc. of mucus). Weakness developed in the hind legs and the dog could hardly walk without assistance. However, on the 9th day of autoclaved yeast administration 1 mg. of histamine gave 19.9 cc. of regular gastric juice (free acidity from 197 to 380% HCl, total acidity from 339 to 485% HCl). On the 10th day the histamine gastric secretion was 30.5 cc., and on the 11th day 5 minutes' sham feeding gave in 40 minutes 25.0 cc. of gastric juice with a much higher concentration of pepsin than in the juice of the third phase or after histamine. At this time the general condition of the animal markedly improved and it could walk unaided. On the 17th day the histamine gastric secretion reached its normal volume, 38.0 cc. The weight of the animal at this time was 15.75 kg. Since our autoclaved yeast was not assayed on pigeons or rats for vitamins B_1 and B_2, and we do not know how much of the former was destroyed in the process, the results reported are merely suggestive. At present we may only state that autoclaved yeast, which presumably contains more vitamin B_2 than B_1, was effective in restoring the normal reaction of the gastric mucosa to nervous and chemical stimuli, although it took a much longer time than non-autoclaved yeast. Vitamins A, D and C do not seem to be essential for the proper functioning of the gastric glands.

7171 P

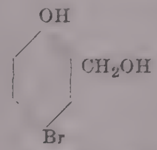
Experimental Pharmacological Study of Mono-Brom-Hydroxy-Benzyl Alcohol.

DAVID I. MACHT AND FITZGERALD DUNNING.

From the Chemical and Pharmacological Research Laboratories, Hynson, Westcott & Dunning, Inc., Baltimore

Macht, Dunning and Stickels[1] described the preparation and some of the pharmacological properties of a large number of derivatives of hydroxy-benzyl alcohol, commonly known as saligenin. Further investigation of the compounds pointed to the mono-brom-hydroxy-benzyl alcohol as being the most interesting of the series, and a more intensive and extensive study of that compound was accordingly begun.

Mono-brom-hydroxy-benzyl alcohol, named *bromsalizol* for convenience, is a white crystalline compound, melting at 107.5°-109°C., which on tasting, produces a numbness of the mucous membranes of the mouth and tongue. Its formula is



with the bromine in the para-position. The analogous compounds with halogen atoms in the ortho- and meta-positions in the benzene ring have also been prepared in our laboratories. Bromsalizol is soluble in water to the extent of 0.5% and is freely soluble in alcohol and olive oil.

When studied on lower and higher animals, the toxicity of bromsalizol was found to be quite low. The minimal lethal dosage for some of the higher animals is shown in Table I. Doses of from 5 to 10 grains (0.3 to 0.6 gm.) have been repeatedly ingested by a large number of men without any discomfort or untoward effect.

The two salient pharmacodynamic properties exhibited by bromsalizol, even when employed in small doses or low dilutions, are (1) its antispasmodic or relaxant effect on smooth muscle tissues and organs, and (2) its local anesthetic effect. Its action on smooth muscle was studied *in situ* on intestines of cats, rabbits and rats, and much more extensively on all kinds of smooth muscle preparations from excised organs of the cat, rabbit, guinea pig, rat, bear, wood-

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[1] Macht, Dunning and Stickels, PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 378.

TABLE I.
Toxicity of Bromsalizol.

Animals*	Method of Administration	Dose per kilo, gm.	Result
Dog (3)	by stomach tube	1.0	fatal
" (3)	intravenous injection	0.25	"
Cat (10)	" "	0.25	"
Rabbit (6)	by mouth	0.5	"
" (6)	intravenous injection	0.25	"
" (3)	" "	0.1	mild stupor; recovery
Guinea pig (6)	intraperitoneal injection	1.0	fatal
Guinea pig (6)	" "	0.2	mild depression; recovery
White rat (10)	" "	0.4	fatal
White rat (10)	" "	0.15	coma; recovery
White mouse (10)	" "	0.5	fatal
White mouse (10)	" "	0.2	coma; recovery

*No. of experiments indicated in parentheses.

chuck and other animals. Experiments on excised muscle strips from the intestines, uterus, gall bladder, urinary bladder, fallopian tubes, ureters and other organs showed that the drug in dilutions of from 1:10,000 to 1:50,000 lowered the tonus of smooth muscle and slowed or completely inhibited the rhythmic contractions without killing the tissue, however, as could be proven by subsequent application of a suitable pharmacological stimulus. (Fig. 1.)

The local anesthetic effects of mono-brom-hydroxy-benzyl alcohol in aqueous or saline solutions were studied by the following methods: (1) instillation into the eyes of rabbits, (2) reflex action of preparations of frogs' legs, (3) the wheal method in guinea pigs, (4) direct application to nerve trunks tested with electric stimuli, and (5) infiltration in different tissues and subsequent surgical operations on the areas infiltrated. It was found that bromsalizol is from 3 to 4 times more efficacious as a local anesthetic than the mother substance, hydroxy-benzyl alcohol, or saligenin.

Action on Vital Functions. Injections of from 5 to 10 mg. of bromsalizol per kilo weight into cats under ether produced no effect on respiration or blood pressure. Doses of from 10 to 25 mg. per kilo weight produced a slight lowering of the blood pressure. Repeated doses of from 25 to 50 mg. of bromsalizol produced marked lowering of blood pressure and a slowing of the respiration, while lethal doses induced death through primary paralysis of the respiratory center. The lowering of blood pressure after moderately large but not lethal doses of the drug is due for the most part to its direct peripheral action, producing vasodilatation. This was established by perfusion experiments and by tests on surviving arterial rings.

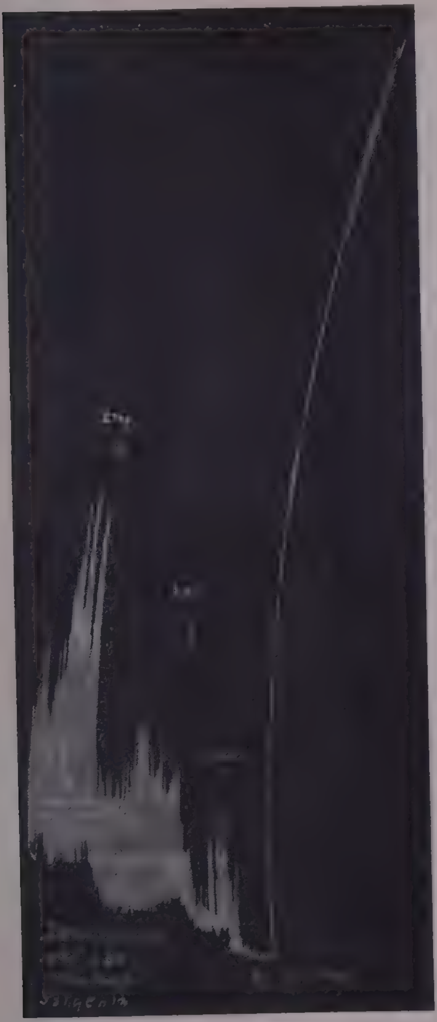

FIG. 1. Loop of small intestine of cat.

Note that 2 mg. of monobrom saligenin relax tonus, and that 6 mg. completely inhibit contractions without killing the preparation, as proven by its subsequent response to 1 mg. of pilocarpine.

Studies on kidney function by the Rowntree-Geraghty phenolsulphonphthalein test and on the liver function by Rosenthal's bromsulphalein method, as well as studies on blood chemistry, after prolonged feeding of the drug to rabbits, indicated that these organs were not injured in any way by even large quantities of the substance.

After having established the maximum tolerated dosage and the toxic or lethal dosage of the compound and after having studied its action on the vital functions of the body, and in consideration of the wide margin of safety between the minimal effective pharmacodynamic and the toxic doses of the drug, carefully controlled clinical observations were made by a number of well-known physicians. Their preliminary reports indicated that the drug was efficacious in relieving spastic conditions, particularly of the gastro-intestinal organs and of the uterus (spastic dysmenorrhea). It was furthermore found by a number of surgeons to be a satisfactory local anesthetic for the performance of minor operations. The favorable preliminary results obtained with the drug render a further and more extensive clinical therapeutic study of the compound very desirable. Full data will be published elsewhere.

7172 C

Ergotoxine Excitement.

ALFRED GILMAN. (Introduced by H. G. Barbour.)

From the Department of Pharmacology and Toxicology, Yale University.

The attention of investigators has been so centered on the peripheral effects of ergotoxine that the central action of this drug has received scant notice. Most of the studies of ergotoxine have been conducted upon anesthetized animals. In these its depressant action upon the respiratory center has been repeatedly reported. There have been few references, however, to central stimulation. Barger and Dale,[1] after intramuscular injection of ergotoxine in cats, observed vomiting and "hyperexcitability". They have recorded similar findings including fever in rabbits. Githens[2] considered this fever to be of central origin. Rothlin[3] found clonic and tonic convulsions 3 hours after large intravenous doses of ergotamine in rabbits. Koppanyi and Evans[4] reported vomiting and defecation of central origin in dogs and cats after the administration of small doses of ergotamine. Recent studies[5] in which ergotamine has been intramuscularly administered to unanesthetized animals in amounts sufficient to affect the sympathetic system make no mention of central action.

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[1] Barger, G., and Dale, H. H., *Biochem. J.*, 1907, **2**, 240.

[2] Githens, T. S., *J. Pharm. and Exp. Therap.*, 1917, **10**, 327.

[3] Rothlin—Cited by Barger, G. Ergot and Ergotism, Gurney and Jackson, London.

[4] Koppanyi, T., and Evans, E., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1181, 1183.

The observations here reported were made upon cats. Unanesthetized animals were tied to boards. No injection was made until the subjects had been calmed. Ergotoxine ethanesulphonate (1 mg./kilo)* was administered by intracardiac injection. This procedure can be accomplished without any excitement of the cat. The immediate manifestations of the action of the drug were those of sympathetic stimulation, wide dilation of the pupil and pilo-erection, especially in the tail. One minute after injection, the central effects became evident. The reflexes were hyperactive, the slightest touch causing a single spastic response in all voluntary muscles. More dramatic, however, were the emotional changes in the animal. The cats previously calm and unmindful of the injection, showed marked rage and apprehension. The slightest stimulation, visual, auditory or tactual, caused a savage hissing and snarling. Objects, brought near the head of the cat were ferociously attacked and bitten. The animals seemed at all times alert and followed all movements closely. Although the hyperactive reflexes and even the rage of the animal might be explained by cortical inhibition, the general demeanor of

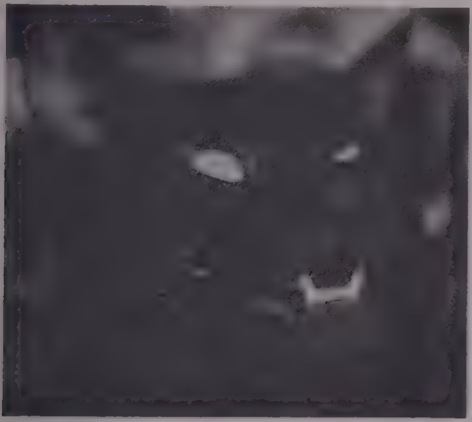

FIG. 1.
Rage During Sympathetic Paralysis.

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[5] Sawyer, M. E. MacKay, and Schlossberg, T., *Am. J. Physiol.*, 1933, **104**, 172.

* Kindly furnished by the Wellcome Research Laboratories, Tuckahoe, New York.

the cat was more suggestive of stimulation. The locus of this action has not yet been fixed.

The syndrome described above persisted for over an hour with diminishing intensity. It is of especial interest that the symptoms of rage were still apparent after sympathetic paralysis. The unusual picture of anger with extremely constricted pupils was presented (Fig. 1). The pupils remained fixed even after the intracardiac injection of large doses of epinephrine (0.4 mg. kilo). The stimulation described above was not followed by any extreme depression. At no time was there any evidence of clonic or tonic convulsions. The above phenomena have been a constant finding in the 12 cats studied.

7173

Vascular Lesions in Hemorrhagic Pancreatitis.

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From the Department of Pathology and Bacteriology, The Johns Hopkins University.

The cause of the extensive hemorrhage which occurs in hemorrhagic pancreatitis has long been a matter of speculation. Diapedesis of red cells from capillaries has been said to occur as a result of the action of trypsin, but no one has demonstrated that trypsin has the power to destroy the walls of previously intact arteries and veins, and in the only histological study of the question which we have been able to find it is stated that the ferment does not attack vessels larger than capillaries.[1] Our microscopical studies of the pancreas in cases of hemorrhagic pancreatitis have revealed the constant and, as far as we can determine, hitherto unrecognized presence of a specific form of necrosis of the walls of arteries and veins, leading to the rupture of the vessels with consequent hemorrhage. We have found the same vascular lesions in experimental hemorrhagic pancreatitis produced by the injection of irritants into the pancreatic ducts of dogs, following the procedure of Opie[2] and Flexner[3]; and we have determined experimentally that this vascular necrosis is the direct result of the action of pancreatic juice upon the walls of the vessels, and that the agent in the juice responsible for the necrosis is trypsin. Pancreatic juice, or pancreatic trypsin freed from lipase, when injected subcutaneously produces in the vessels at the site precisely the same vascular necrosis with hemorrhage as that which occurs regularly in hemorrhagic pancreatitis, and when trypsin is injected into the pancreas hemorrhagic pancreatitis results. The activation of trypsinogen by enterokinase is not necessary for the production of the vascular necrosis, for pancreatic juice obtained by cannulating the pancreatic duct outside the duodenum produces local vascular necrosis and hemorrhage when injected into the subcutaneous tissue. We have not yet determined precisely what it is that serves to activate trypsinogen when it is injected into the tissue. It is, however, clear from our experiments that if pancreatic juice escapes about blood-vessels in the substance of the pancreas, necrosis of the vessels with hemorrhage (hemorrhagic pancreatitis) can occur.

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[1] Achalme, *Ann. Inst. Past.*, 1901, **15**, 737.

[2] Opie, *Johns Hopkins Hosp. Bull.*, 1901, **12**, 182.

[3] Flexner, *Univ. of Pennsylvania Med. Bull.*, 1901-2, **14**, 193.

Study of the pancreas in our unselected routine autopsy material has revealed an unexpected frequency of duct obstructions caused by concretions and by the type of epithelial metaplasia described by Baló and Ballon.[5] Localized dilatations of the ducts and acini behind these obstructions, and the rupture of the thin-walled, dilated acini is not at all uncommon in our routine material. If the juice escaping from such ruptured acini flows about arteries, necrosis of their walls with resulting hemorrhage may occur, leading to widespread or to localized hemorrhagic pancreatitis depending upon the size of the vessels affected and the degree to which their walls are damaged by the escaping trypsin. If no arteries are present in the immediate vicinity of the rupture, localized fat necrosis, produced by the lipase, may be the only result, and this is most frequently the case in our material. That hemorrhagic pancreatitis can result from certain conditions which do not involve duct obstruction there is no question, but we believe that the rupture of acini dilated as a result of obstruction plays a rôle in the pathogenesis of hemorrhagic pancreatitis more frequently than has been appreciated. We have found histological evidence of obstruction in nearly 50% of our cases of hemorrhagic pancreatitis.

It is of interest that by means of the local injection of trypsin or of pancreatic juice we have been able to produce in the arterioles of the pancreas and of the subcutaneous tissue a lesion which is identical histologically with the familiar hyaline arteriolosclerosis which, in the human being, commonly occurs diffusely in association with hypertension and arteriolosclerotic nephritis. We are attempting to determine whether generalized hyaline arteriosclerosis can be produced by repeated injections of trypsin, and whether the proteolytic enzymes of bacteria are able to cause this lesion.

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[5] Baló and Ballon, *Arch. Path.*, 1929, 7, 27.

7174 C

Spinal Anesthesia in Winter Frogs.*

RAYMOND N. BIETER AND JAMES W. BROWN.

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In a previous communication[1] a simple method for producing spinal anesthesia in the frog was described. In addition, a series of 10 local anesthetics were studied in summer frogs, kept at temperatures of from 26° to 29°C. The data obtained on each drug included the determination of the minimal anesthetic dose, minimal lethal dose, duration of anesthesia produced by a sublethal dose, and the calculation of a therapeutic coefficient from the fraction M.L.D./M.A.D. Comparatively, this coefficient gives evidence of the usable therapeutic range for each local anesthetic.

In this paper similar results are described in which 7 local anesthetics were injected intraspinally into winter frogs kept before, during and after the experiments at 10° to 12°C. This series includes those local anesthetics showing, experimentally, the best possibilities for use in human spinal anesthesia. Five of these drugs had been studied previously in summer frogs, but because of the relatively high temperature prevailing at that time, it was thought advisable to repeat the work at a lower temperature and perhaps a more satisfactory one for frogs. Because of great variations noted in frogs, which are ascribed to seasonal and temperature changes, a further check on uniformity was attempted by carrying out the complete series during two winters. Consequently the first series was carried out between October 10, 1931, and February 20, 1932, and the second, between the same periods of 1932-33. As can be seen from the table no great variations are to be observed between the 2 series. It might also be added that the results obtained on winter frogs

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* This work was aided by a grant from the Graduate School Research Fund of the University of Minnesota.

[1] Bieter, R. N., Harvey, A. M., and Burgess, W. W., *J. Pharm. and Exp. Therap.*, 1932, **45**, 291.

show a close parallel to similar data obtained on the rabbit. These will be reported later.

The method of experimentation used was identical with that described previously. The % solutions used were as follows:

For determining	M.A.D.	M.L.D.
Nupercaine	.3	.6
Pantocaine	.15	1.
Metycaine†	.5	2.
Larocaine	.5	10.
Panthesine	.5	10.
Tutocaine	.5	10.
Procaine	2.	10.

† Metycaine was formerly called neothesine.

These variations were necessary in order to meet the requirements of a small uniform volume for all injections. The criterion for determining the M.A.D. was the same as before, that for the M.L.D. differed in one respect, namely, that the time interval for determining death had to be lengthened from 3 hours, as used in summer frogs, to 24 hours. A total of 850 male frogs was used, obtained fresh every week and contained in a running water tank for 2 to 3 days before use.

TABLE I.

Minimal Anesthetic and Minimal Lethal Doses, and Therapeutic Coefficients; intraspinal anesthetics in winter frogs

Drug	M.A.D. in mg. per gm. wt.	Aver. of 2 series	M.L.D. in mg. per gm. wt.	Aver. of 2 series	Ratio: M.L.D./M.A.D. (Therapeutic Coef.)
Nupercaine	.006* .006	.006	.008 .008	.008	1.3
Procaine	.045 .045	.045	.28 .30	.29	6.4
Metycaine	.01 .01	.01	.075 .075	.075	7.5
Pantocaine	.004 .003	.0035	.035 .03	.0325	9.2
Larocaine	.0125 .0125	.0125	.155 .155	.155	12.4
Panthesine	.0075 .0065	.007	.15 .145	.147	21.0
Tutocaine	.015 .015	.015	.35 .35	.35	23.3

* Upper figure, 1931-32 series, lower, 1932-33.

Table I shows the M.A.D., M.L.D., and therapeutic coefficient for each of the 7 anesthetics. The 5 drugs used in summer frogs showed a marked drop in toxicity at the lower winter temperature. These diminutions in toxicity in per cent of the summer M.L.D.'s were as follows: Nupercaine 100%, procaine 31%, metycaine 50%, pantocaine 62%, and tutocaine 75%. On the other hand the M.A.D.'s were practically the same with the exception of nupercaine which was almost 50% less active at the lower temperature.

Summary. The three local anesthetics, larocaine, panthesine and tutocaine, which chemically are of the procaine type, but with branched side chains, gave the highest therapeutic coefficients. In a decreasing order of sensitivity (based on the M.A.D.) the anesthetics can be arranged as follows: pantocaine > nupercaine > panthesine > metycaine > larocaine > tutocaine > procaine. In an increasing order of toxicity (based on the M.L.D.) the anesthetics can be arranged as follows: tutocaine < procaine < larocaine < panthesine < metycaine < pantocaine < nupercaine.

Grateful appreciation is accorded to the following firms: The Ciba Company, Inc., Eli Lilly and Company, Sandoz Chemical Works, Inc., Hoffmann-LaRoche, Inc., and The Winthrop Chemical Company, Inc., for generously supplying us with the nupercaine, metycaine, panthesine, larocaine, and procaine (novocain) and pantocaine, respectively, used in these experiments.

7175 P

Life History of Manson's Blood Fluke (Schistosoma mansoni). I. Extramammalian Phase of the Cycle.*

ERNEST CARROLL FAUST AND WILLIAM A. HOFFMAN.

From the Department of Tropical Medicine, Tulane University, and the School of Tropical Medicine, San Juan, P. R.

The work of which this contribution forms a part was carried on in Puerto Rico, where Manson's blood fluke infection is an important and extensive clinical entity. The lateral-spined eggs of the parasite were obtained from the feces of human and experimental animals. The stools were washed and concentrated either by centrifugation or by sedimentation. The numbers of these eggs obtained from a 24-hour specimen varied not only with the age of the infection in the mammalian host but also fluctuated from day to day. The majority were viable, and contained ciliated larvae (miracidia), which were studied for detailed morphological features, including the number and pattern of the epithelial plates.

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* Supported by grants from the National Research Council and the Bailey K. Ashford Scholarship Fund.

Hatching was found to be due to several factors, including the maturity and vitality of the miracidium, the tonicity of the medium and mechanical agitation of the eggs. Some larvae hatch immediately after washing and concentration but the majority require from 12 to 24 hours. After swimming around for several minutes in or near the bottom sediment the miracidia rise to the top of the water level and swim energetically in the top inch of the water. Under laboratory conditions their length of free-living existence was not over 24 hours, usually considerably less. If specimens of laboratory bred snails of the species *Helisoma (Planorbina) guadeloupense*, the appropriate intermediate host in Puerto Rico, are placed in a pan containing miracidia hatched from washed viable eggs of *S. mansoni*, they are soon attacked by the miracidia, which are attracted to the soft parts of the snail, particularly the tentacles. Some of these larvae secure a permanent hold on the tissues and bore their way inward. The compatibility of this host-parasite relationship was confirmed by the high percentage of snails which usually became infected (75-100%). Exposed snails were sacrificed at suitable intervals and dissected to determine the stage of development of the intramolluscan phase and the position of the parasite in the snail's body. Primary sporocysts were first isolated and identified on the eighth day (in a circumesophageal lymph sinus). From that time on development was readily traced through the propagation of second generation sporocysts and cercariae (larvae of the third generation). Complete development, with spontaneous rupture of the cercariae, was found to require 24-28 days in spring and summer and 30-35 days in winter. Infected snails are much more likely to succumb to unfavorable conditions of the environment than are uninfected ones. Most snails become infected with only a few miracidia. The cercarial progeny arising from a single miracidium may be discharged over a period of 4 months or longer and may amount to more than one hundred thousand individuals. The greatest daily discharge occurs about 2 months after the first cercariae mature.

The free-living cercariae are phototactic and emerge from their snail hosts commonly between 9 o'clock in the morning and 1 o'clock in the afternoon, particularly when exposed to direct sunlight. At first they are usually assembled as a "halo" around the opening of the snail's shell. Later, up to 8-10 hours, they tend to become more or less equally distributed through the water, apparently poised in a vertical position, with tails almost always upward, but actually moving very slowly in the current of water. From time to time they actively swim about, with their anchor-flukes (bifid tail) directed forward, but in a few seconds resume their passive pose. They readily become entangled in mucus discharged by the snail. This distribution through the water is maintained for 18-24 hours, after which they more commonly crawl about on the bottom of the container, but can be readily activated. They move away from moderate cold but if placed in the ice box will sink to the bottom of the container and survive for 24 hours or longer at or just above the freezing temperature. On return to normal temperature (22-30°C.) they readily become activated. Less than 5% die within the first 24 hours but by 30 hours 90% have perished.

The cercariae of *Schistosoma mansoni* are found on careful examination of immature and mature living specimens to have *two pairs of anterior secretory (penetration) glands* with large dense granular contents and *4 pairs of posterior secretory glands* with finely granular contents. In a small percentage of specimens freshly emerged from the snail evidence of a head gland has been found.

The cercaria is the infective stage for man or other mammals which wade, bathe, swim or otherwise expose their skin to the "infected water".

7176 P

Life History of Manson's Blood Fluke (Schistosoma mansoni). II. The Mammalian Phase of the Cycle.*

ERNEST CARROLL FAUST, CHARLES A. JONES AND WILLIAM A. HOFFMAN.

From the Department of Tropical Medicine, Tulane University, and the School of Tropical Medicine, San Juan, P. R.

In Puerto Rico man is the only known definitive host of *Schistosoma mansoni* infection. The experimental data included in this report are based on infections of laboratory rats and rabbits and rhesus monkeys, as well as on one accidental human infection. Twenty-three rats, 8 rabbits and 4 monkeys were submitted to inoculation, either by placing the animal for one to 2 hours in a bath of water swarming with cercaria, or by placing desirable food in such water, or by spraying infected water into the animal's mouth. Evidence that cercariae were attacking the animal's skin within an hour after exposure was furnished by the extreme nervousness and later by intense pruritus of the exposed tissue of the animal. This continued for several hours after the animals were removed from the infective bath. All animals exposed became infected. The animals were sacrificed under anesthesia at intervals varying from 16 hours to 70 days, the cavities were opened, the various organs and tissues carefully inspected for gross evidence of infection and then the several organs were separated by ligation and removed from the body into isotonic citrate solution. These tissues and organs were thoroughly perfused and/or chopped up and the solution centrifuged and then examined for migrating larvae. Likewise, suitable portions of each organ were fixed in Zenker's fluid and sectioned. By these methods it was found possible to trace the route of migration and development of the young worms day by day through the prepatent period.

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*Supported by grants from the National Research Council and the Bailey K. Ashford Scholarship Fund.

In general the development follows that described by various workers for *Schistosoma japonicum*, the Oriental blood fluke, but with important exceptions. The migration route is as follows. Entry of the cercariae through the skin takes place within a few hours after exposure. Within 16-20 hours after inoculation the larvae have left the skin, most usually *via* the peripheral veins, but at times passing through peripheral lymph nodes. They are carried through the right heart to the lungs, where they may be found in the pulmonary capillaries as early as 20 hours and in the peribronchial venules up to 8 days. However, some get through to the left heart as early as the 3rd day and pass into the systemic circulation. As early as the fourth day a few larvae have been recovered from the intra-hepatic portal veins, with evidence that in this location they feed on blood for the first time. But they do not remain there; rather, they pass through the portal filter and back through the right heart to the lungs, where fed larvae are found up to the 19th day. Likewise, there is no evidence that larvae in the general circulation become filtered out in the stomach wall, kidneys, lymph glands, etc. They work their way through these filters, pass again through the lungs, and eventually (about the 13th day) accumulate in the liver. The larvae migrate passively from the pulmonary venules *via* the left heart, aorta and mesenteric arteries into the portal system. In no single instance was there any indication that young larvae which had gotten out of the lungs into the pleural cavity were capable of or ever attempted to penetrate through the diaphragm into the peritoneal cavity, and by this route enter the portal system.

The stages of development parallel those of *S. japonicum* but there is a constant "lag" of 5-10 days in *S. mansoni.* Sexual maturity in *S. mansoni* is reached between the 35th and 40th day, although even in light infections only a fraction of the worms are mature and the females laying eggs before the 40th day. *Schistosoma japonicum,* when mature, has a predilection for the upper branches of the superior mesenteric vein (*i. e.,* arising from the jejunum and upper ileum). On the other hand, *S. mansoni* migrates into the lower branches of the superior mesenteric vein and the inferior mesenteric vein, to deposit eggs in the venules of the wall of the colon. In a monkey sacrificed on the 54th day mated worms were also found in numbers in the superior and middle hemorrhoidals and even in the inferior vena cava. Furthermore, eggs-laying pairs have on several occasions been found in sections of lung tissue (rats, rabbit, monkey).

Series of adult females were examined to determine the numbers of eggs *in utero.* There is usually only one egg present but in about 8% of 355 females studied 3 or more eggs were seen. However, evidence provided by egg counts of representative samplings of the bowel mucosa indicates that each female lays several tens of eggs *per diem,* although eggs evacuated in the feces account for only a small fraction of those laid, the remainder having infiltrated into the tissues of the bowel wall.

7177 P

Life History of Manson's Blood Fluke (Schistosoma mansoni). III. The Blood Picture in Schistosomiasis Mansoni.*

ERNEST CARROLL FAUST AND CHARLES A. JONES.

From the Department of Tropical Medicine, Tulane University, and the School of Tropical Medicine, San Juan, P. R.

The blood picture in experimental mammals infected with *S. mansoni* shows first a leukocytosis, paralleling the migration of the larvae through the lungs. This is followed by a gradually develop-

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* Supported by grants from the National Research Council and the Bailey K. Ashford Scholarship Fund.

ing leukopenia, with or without an eosinophilia, which, if present, is most pronounced about the end of the prepatent period. From about the 40th day there is an excess of euglobulin in the blood plasma. Moderate to late clinical cases usually give a positive precipitation test for serum globulin. The total erythrocyte counts are little altered in experimental and clinical cases, but there is definite evidence of regeneration of these cells, as indicated by a hyperplastic bone marrow and an increased reticulocyte count.

7178 P

Functional Capacities of Ovaries of New-born after Transplantation into Adult Ovariotomized Rats.*

CARROLL A. PFEIFFER. (Introduced by E. Witschi.)

From the Zoological Laboratory, State University of Iowa.

Smith and Engle[1] pointed out that in mice more than 10 days old, sexual maturity may be induced by hypophysis transplants, but at this early date 5 daily transplants are required. After the 17th day 2 or 3 daily transplants are sufficient. Hill[2] has shown that this age difference holds for injections of pregnancy urine. However, the ovary of the new-born rat responds neither to the amount required for the 15-20-day-old rat nor to a much higher dosage (Wiesner[3] and Pfeiffer, unpublished work). It remained to be tested how early the ovary of the new-born might mature if it were subjected to gonadotrophic hormones from the time of birth. This could be done by injections. However, if the ovaries are transplanted into an adult female at the time of castration, they will be supplied with a constant, or possibly a steadily increasing amount of anterior pituitary hormone.

Young, healthy, mature female rats whose cycles had been followed for some time previous to the operation were used for hosts. The ovaries were obtained from new-born young and one ovary was transplanted under the dorsal surface of each kidney of the host, on which bilateral ovariotomy was performed at the time of implantation. Seven animals were used and the grafting was successful in 5 cases. The changes in the ovary were followed by daily vaginal smears which also indicated the extent to which the immature ovary was able to fulfill the endocrine functions of the adult ovary. After 4½ to 8½ months the grafts and the hypophyses were removed, sectioned, and studied, in order to correlate the oestrus condition with the histological structure in the ovary and in the hypophysis. Proof that no regeneration of the ovaries had taken place was obtained by a study of sections through the region of the ovarian stalk and by comparison with a series of control castrates.

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* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Emil Witschi.

[1] Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, **40**, 159.

[2] Hill, Margaret, *J. Physiol.*, 1932, **75**, 44.

[3] Wiesner, B. P., *J. Physiol.*, 1932, **75**, 39P.

Following ovariotomy the cycles immediately cease, but if the graft takes, they reappear after 12 to 16 days. The return of sexual activity begins with a rather long oestrus stage of 5 to 8 days which is followed by fairly regular but usually slightly prolonged cycles. The average duration of the cycles is 5½ to 6 days instead of the usual 4 to 4½ days. The lengthening occurs either in the cornified or the leucocyte stage, extending to 10 days in a few cases. The oestrus cycles of one of the rats were as regular as those of the controls except that they were consistently of 5½ day intervals. The cycles of a second animal ceased after a short time and biopsy showed only one graft, which was also undergoing degeneration. A third animal, after about 3 months of cyclic function, ran constant oestrus until autopsy 4 weeks later.

The ovarian grafts are quite large due to large cystic follicles, which in some cases are 1.5 cm. in diameter. This indicates that ovulation does not take place. The walls of the large cystic follicles are composed of connective tissue rather than follicle cells. Only a few ripening follicles and a few remnants of corpora lutea are found, yet some of the hosts show fairly normal cycles even in the absence of both ripe follicles and corpora lutea. As shown by Levine and Witschi[4] the absence of follicles does not necessarily preclude cyclic function. Therefore, the follicles are evidently not the only factors which bring about oestrus. Since corpora lutea may be absent, it is apparent that they are not necessary for the return to dioestrus.

In one animal, autopsied after 4 weeks of constant oestrus, the graft contained several cystic follicles and a nearly full grown follicle in which was found a degenerating ovum with a first maturation spindle. Castillo[5] has reported that ripe follicles and corpora lutea are found in ovarian grafts (immature ovaries) in castrate females but that there is "no tumultuous initiation of cycles and the grafts have only a short duration." In the present experiment the grafts were able to maintain the cyclic functions for 8½ months, at which time the animals were autopsied.

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[4] Levine, W. T., and Witschi, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1152.

[5] del Castillo, E. B., *Compt. Rend. Soc. Biol.*, 1932, **109**, 331.

In only one case did the influence of the graft completely prevent formation of castrate cells in the hypophysis. In fact, in the animal which had been in constant oestrus for some time before autopsy, the hypophysis showed quite a large number of castrate cells. Haterius and Nelson[6] have shown that the mature ovarian graft is able to bring back the normal histological picture in the hypophysis of the male as well as in the female. However, our results show that the grafts of the ovary of the new-born do not as a rule maintain the normal condition of the hypophysis, even though they do maintain the cyclic changes in the genital system. The secondary sex characters are maintained, and the animals show varying amounts of sexual drive. They copulate quite frequently at the beginning of the cornified cell stage.

It is concluded from this experiment that the ovary of the new-born rat matures under the influence of the adult (castrate) hypophysis within 12 to 16 days, after which time it is able to maintain the cyclic changes in the genital system. However, due to the abnormal conditions of the transplantation, the ovary is able neither to ovulate nor to insure maintenance of the normal histological picture of the hypophysis. Ripe follicles and corpora lutea are not indispensable to cyclic function of the ovary.

7179 C

Observations Upon Refractory Period of Auricular Extrasystoles in the Mammalian Heart.

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From the Cardiographic Laboratory, Department of Medicine, Johns Hopkins Hospital and University.

By means of an adaptation of the method reported by Andrus and Carter,[1] the authors have determined the refractory period of extrasystoles induced at various points in the cardiac cycle in the normally beating dog's auricle. A typical experiment in which extrasystoles were induced soon after the end of the refractory period of the spontaneous excitation is summarized in Table I.

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[6] Haterius, H. O., and Nelson, W. O., *J. Exp. Zool.*, 1932, **61**, 175.

* Jacques Loeb Fellow in Medicine.

[1] Andrus, E. Cowles, and Edward P. Carter, *J. Exp. Med.*, 1930, **51**, 357.

TABLE I.

	Before Adrenalin				After Adrenalin			
	Spontaneous beat		Extrasystoles produced .1470 sec. ±.0031 sec. after spontaneous beat		Spontaneous beat		Extrasystoles produced .1160 sec. ±.0053 sec. after spontaneous beat	
Columns	I	II	III	IV	V	VI	VII	VIII
Auricular rate	136		133		218		219	
Response	.1526	.1509	.1306	.1179	.1251	.1201	.1213	.0848
	.1460↓	.1446↑	.1226↓	.1138↑	.1104↓	.1159↑	.0931↓	.0800↑
	.1394	.1367	.1112	.1092	.0978	.1083	.0799	.0746
Refractory period	——	——	——	——	——	——	——	——
No response	.1336	.1338	.1034	.1044	.0900	.0922	.0708	.0716
	.1222↓	.1291↑	.0907↓	.1000↑	.0800↓	.0820↑	.0663↓	.0640↑
	.1209	.1267	.0925	.0923	.0665	.0723	.0620	.0617

↓ with decreasing and ↑ with increasing intervals. R-S_1 and S_1-S_2 intervals in seconds.

Columns I and II depict the determination of the refractory period of the spontaneous beat. The intervals, which are given in seconds, were measured between a spontaneous excitation and the induced stimulus which followed (R_1-S_1 interval). Those stimuli represented above the "refractory period" line were followed by auricular extrasystoles; those below were not. The refractory period, therefore, lay between the shortest interval which was followed by a response and the longest which was not.

Each series is made up of consecutive observations; those of Column I with successively decreasing, and those of Column II with successively increasing, R-S intervals. Identical supra-maximal stimuli were used throughout.

Columns III and IV illustrate the estimation of the refractory period of extrasystoles induced 0.1470 sec. ± 0.0031 sec. after spontaneous excitations. The intervals are those between a stimulus which produced an extrasystole and a succeeding identical stimulus (S_1-S_2 interval). The stimuli above the "refractory period" line were followed by a second auricular systole, those below were not. The refractory period of the auricular extrasystoles, therefore, lay between the extremes as defined above. These results are also from consecutive observations with successive S_1-S_2 intervals shorter in column III and longer in column IV.

Columns V, VI, VII and VIII are similar to columns I, II, III and IV, respectively, and give the results of observations made upon the same preparation during the constant intravenous infusion of adrenalin (1-25,000).

In this experiment the refractory period of normal auricular excitations was found to lie between 0.1338 sec. and 0.1367 sec. The refractory period of extrasystoles induced 0.1439 sec. to 0.1501 sec. after normal excitations was between 0.1044 sec. and 0.1092 sec. The same ratio obtained when the cardiac rate was accelerated by the constant intravenous infusion of adrenalin. The refractory period of normal excitations lay between 0.0922 sec. and 0.0978 sec. while the refractory period of early extrasystoles was between 0.0716 sec. and 0.0746 sec.

In a series of 19 animals it has been demonstrated that the refractory period of a premature systole is considerably (20-30%) shorter than that of a normal spontaneous excitation. Moreover, the earlier the extrasystole is induced in the normal diastole, the shorter is its refractory period.

Complete results of the other observations, and the significance of these findings with relation to certain ectopic rhythms, will be discussed in a subsequent communication.

7180 P

Reversible Inactivation of Bacteriophage by KCN.

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From the Department of Bacteriology, University of California, Berkeley.

It has recently been shown that the complete inactivation of phage following treatment with $HgCl_2$ can be reversed by precipitation of Hg^{++} with restoration of the phage to its original titre.[1, 2] The phenomenon is interesting in that reactivation occurs after exposure to very concentrated $HgCl_2$ solutions for long periods of time, conditions which produce irreversible loss of the growth function in even the most resistant spore forms of bacterial life. The suggestion was advanced[2] that this behavior spoke for the enzyme concept of phage rather than for the view that phage is composed of living protoplasm.

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[1] Krueger, A. P., and Baldwin, D. M., *J. Gen. Physiol.*, 1933, **17**, 129.

[2] Krueger, A. P., and Baldwin, D. M., *J. Gen. Physiol.*, 1933, in press.

Further work seemed indicated to determine whether the reversible inactivation of phage with $HgCl_2$ represented a special case or whether, as seemed likely, it was merely one example of a general property of phage. We wish to summarize here certain experiments on the cyanide-phage reaction.

1. *Inactivation.* Standard antistaphylococcal phage[3] suspended in beef infusion broth of pH 7.0 was mixed with an equal volume of 1/5,000 KCN (M/325) solution. The mixture was kept at room temperature for 24 hours after which time the following samples were taken: (a) 1 ml. immediately diluted 1/1,000 with broth for determination of residual [P]. (b) 10 ml. for reactivation.

2. *Reactivation.* To 10 ml. of inactivated phage was added 1 ml. of 2.74% $AgNO_3$ and 2 ml. of 1% KCN, the purpose being to remove the CN ions from their union with phage and to combine them in the complex double ion $Ag(CN)_2$. The latter ion does not form insoluble compounds with the cations present and therefore obviates the dangers of phage adsorption on a precipitate. Dilutions of 1 part of the reactivated mixture to 1000 parts of broth were made at once for subsequent titration.

3. *Controls.* (a) Equal volumes of various phage concentrations and 1/5,000,000 KCN (M/325,000) solution were mixed together. These solutions were titrated along with the inactivated and reactivated series to detect any possible influence of 1/10,000,000 KCN upon the titration system. (b) The reactivated solutions themselves served as an adequate means of observing the effects of $Ag(CN)_2^-$ ions on the titration set up. It was found that neither the CN^- ions nor the $Ag(CN)_2^-$ ion in the concentrations employed altered the Bacterium-Bacteriophage reaction utilized in the quantitative determination.

4. *Titration.* All titrations were performed according to the procedure developed by Krueger for the quantitative determination of phage.[3, 4] In this method an activity unit having the general dimensions of a velocity constant is employed and as in certain enzyme determinations the results of each individual experiment are referred to a standard solution (containing in phage work 1×10^{10} such units).

Results. In 4 separate experiments it was found that the inactivation of phage by exposure to M/650 KCN for 24 hours could be completely reversed by converting CN^- to $Ag(CN)_2^-$. The yields were respectively 78%, 100%, 80%, and 98%, of the initial phage.

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[3] Krueger, A. P., *J. Gen. Physiol.*, 1929, **13**, 557.

[4] Krueger, A. P., *Science*, 1930, **72**, 507.

While inactivation in these experiments appears to be complete the method of carrying out the test involves the possibility of a small residuum of active phage escaping detection, for the inactivated solution is diluted 1:1000 with broth before titrating it. This step is necessary in order to bring the KCN concentration down to a level (1/10,000,000) which will not interfere with the titration reaction. However, even should some phage not be inactivated the maximal theoretical amount that might be overlooked would be very small indeed; at most not more than two hundred thousandths of one per cent of the original phage titre.

Conclusions. 1. The inactivation of antistaphylococcal bacteriophage suspended in infusion broth at pH 7.6 by CN^- can be completely reversed by converting CN^- to $Ag(CN)_2^-$. 2. The previously reported reversible inactivation of phage by Hg^{++} therefore appears to be not a special case but rather one example of a more or less general property of phage.

7181 P

Effect on Total Thyroidectomy on Response to Adrenalin.

EUGENE C. EPPINGER AND SAMUEL A. LEVINE. (Introduced by E. C. Cutler).

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The following observations have been carried out as a part of the study concerning the therapeutic value of total thyroidectomy in angina pectoris. It was noted a few years ago[1] that intramuscular injections of adrenalin, when given to patients suffering with angina pectoris would provoke attacks of anginal pain. As a result of clinical observation, it was found that when total thyroidectomy was performed in patients who were having daily attacks of angina, these attacks disappeared immediately following the operation and long before any changes had taken place either in the basal metabolism or in the rate of blood flow. It was therefore suspected and, in fact, predicted[2] that removal of the thyroid gland might be producing a fundamental alteration in the response of the circulation to adrenalin and that a previously positive response might possibly become negative after such an operation.

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[1] Levine, S. A., Ernstene, A. C., and Jacobson, B. M., *Arch. Int. Med.*, 1930, **45**, 191.

[2] Levine, S. A., Cutler, E. C., and Eppinger, E. C., *New Eng. J. Med.*, 1933, **209**, 667.

Five patients were given intramuscular injections of adrenalin varying from 0.3 to 0.5 cc. of a 1-1000 solution before and 2-3 days after operation. In the first of these cases, which was operated upon August 22, 1933, 0.5 cc. before the operation induced a maximum rise of the pulse rate of about 20 and the blood pressure rose from 188/88 to a maximum of 226/94 in a few minutes. Typical substernal anginal pain also resulted. On August 23, 1933, 0.5 cc. of adrenalin produced no pain, a rise in the pulse rate of only 10 and the blood pressure was hardly changed. On August 26 0.5 cc. of adrenalin again produced no chest pain, although the systolic blood pressure rose about 30 mm. On August 27, 1.0 cc. of adrenalin again failed to produce anginal pain and the effect on the pulse and blood pressure was slight. Here, although twice the original amount of adrenalin was given, the effect on the pulse rate and blood pressure was less and there was no chest pain.

In a second case, preoperatively 0.5 cc. of adrenalin produced a rise in the pulse rate of about 18 with no change in the blood pressure. Definite anginal pain resulted. Three days after the operation the same dose of adrenalin caused no pain nor appreciable changes on the pulse rate or blood pressure. In a third case, 0.4 cc. preoperatively produced severe anginal pain with a rise in the pulse rate of about 35 and a change in the blood pressure from 140/85 to 165/95. Two days after operation, the same dose of adrenalin caused no anginal pain although the pulse rate and blood pressure increased almost as much as before. In the fourth instance, slight anginal distress was produced by 0.4 cc. before operation and the pulse rate and blood pressure showed moderate increases. Two days after the operation a similar amount of adrenalin produced no chest distress whatever and very slight elevations in pulse rate and blood pressure. In a fifth case, 0.3 cc. of adrenalin reproduced typical anginal pain. The pulse rate rose slightly and the blood pressure moderately. Two days after operation the same dose of adrenalin caused practically no change in the pulse rate but the blood pressure rose moderately. Definite anginal pain resulted, however, but the patient stated that the distress was much less than on the previous occasion.

Preoperatively, the average basal metabolism of these 5 patients was —4% and the rate of blood flow, 18 seconds, whereas 2 to 3 days after the operation the corresponding figures were +4% and 19 seconds. Further observations on the effect of adrenalin in our patients with angina pectoris were discontinued because of the hazard involved. More detailed studies along this same line are, therefore, being carried out in animals.

This study suggests that the complete removal of the thyroid gland alters the response of the cardiovascular system to adrenalin and that this effect is independent of changes in the basal metabolism or the rate of blood flow. We also believe that it is the alteration of this mechanism which may in a measure be responsible for the relief of anginal pain in these patients.

7182 C

Loss of Sensitivity to Anterior Pituitary-like Hormone of Pregnancy Urine.

H. SELYE, J. B. COLLIP and D. L. THOMSON.

From the Department of Biochemistry, McGill University, Montreal.

It has been shown that the greatly enlarged ovaries of rats injected with extract containing the anterior pituitary-like hormone of pregnancy urine (A.P.L.) will decrease in weight after a certain period even though administration of the hormone be continued. Such ovaries will finally return to a normal weight level.[1, 2, 3]

We have recently repeated these experiments on a series of 12 rats, continuing the A.P.L. injections for a longer time, and we found that after prolonged administration of the hormone the ovaries of some of these rats became actually subnormal in size, weighing as little as 7 mg. after 4 months' treatment (increasing doses of 25-200 units of A.P.L. daily). The ovaries of 8 out of 10 rats, however, were still normal in size at this time (30-45 mg.), but they lost their sensitivity to A.P.L., for 200 units per day of this hormone did not have any luteinizing effect upon them. Histologically they showed numerous corpora lutea in retrogression, but no mature follicles.

In 4 similarly treated rats we performed a laparotomy, inspected the ovaries after 4 months of treatment with A.P.L. and found them normal or subnormal in size. We then injected these rats over a

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[1] Collip, J. B., *Internat. Clinics*, 1932, **4**, 51.

[2] McPhail, M. K., *J. Physiol.*, 1933, **80**, 105.

[3] Zondek, B., *Die Hormone des Ovariums und des Hypophysenvorderlappens*, Julius Springer, Berlin, 1931.

period of 14 days with an aqueous ammoniacal gonadotropic extract of pig pituitary in addition to A.P.L. At autopsy all of these ovaries were greatly enlarged and contained numerous fresh corpora lutea. Their weights ranged from 99-170 mg.

These experiments show that even if the ovary loses its sensitivity to the anterior pituitary-like hormone of pregnancy urine, it still responds readily to extracts obtained from the hypophysis.

7183 C

Influence of the Uterus on Ovary and Mammary Gland.

HANS SELYE. (Introduced by J. B. Collip.)

From the Department of Biochemistry, McGill University, Montreal.

While Caesarian section normally initiates lactation in the rat, it has been found that milk secretion does not occur if the uterus is filled with paraffin immediately after the foetuses have been removed.[1] Furthermore, no oestrus is usually observed in such rats for several weeks after the operation; while in the controls, the uteri of which have not been distended, normal cycles set in 3 days after the interruption of pregnancy. We have recently repeated these experiments, and it is the object of this paper to describe the morphological changes observed in the ovary, pituitary and mammary gland.

In 10 pregnant rats, the uteri were emptied through a small incision during the second half of gestation; then each horn of the uterus was injected separately with melted paraffin (melting point 42°C.) after tying the lower end with silk. When the uterus was well distended, the upper end, through which the injection was made, was also tied.

During the 6 weeks following the operation, only 2 of these animals came into oestrus—one had 2 cycles, the other, 4. At autopsy it was found that the ovaries were greatly enlarged (75-110 mg. as compared with 40-45 mg. in the normal controls), and contained many large corpora lutea similar in appearance to those observed during gestation (Fig. 1).

In a control series where the foetuses were removed, but the uterus was not filled with paraffin, the corpora lutea of gestation shrank

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[1] Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinology*, in press.

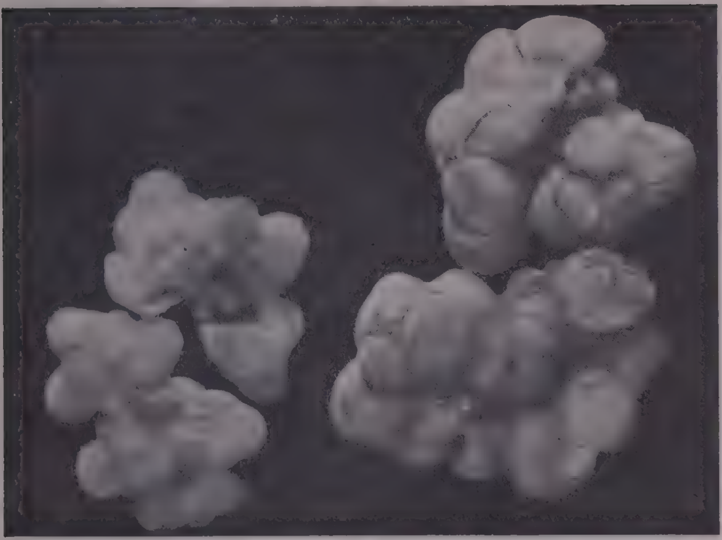

FIG. 1.

To the left, ovaries of normal control rat; to the right, ovaries of a rat 6 weeks after foetuses have been replaced by paraffin. Note the large corpora lutea similar to those present during gestation.

rapidly after the operation, and were as small as the corpus luteum of a normal cycle within 6 days after operation.

Histologically, the corpora lutea of the experimental animals consisted of cells rich in cytoplasm with a large vesicular nucleus; they showed no signs of round cell infiltration or of invasion by connective tissue, and resembled closely the corpora lutea of gestation. Sections stained with Sudan III showed, however, that besides the small lipoid granules characteristic of the active corpus luteum of gestation, larger fat globules appear in many of the cells. In none of these ovaries did we find mature follicles.

The pituitary was greatly enlarged in all these rats, weighing 11-17 mg. as compared with 7-8 mg. in the normal controls.

From these findings it appears that uterine influences may inhibit the involution of the corpus luteum of gestation, the maturation of follicles and oestrus in the rat. It is interesting to note, however that the mammary glands of these animals have not been maintained in the pregnancy stage, but have involuted completely.

We found, furthermore, that distention of the uterus with paraffin will also inhibit post-partum milk secretion. Ten lactating rats, the uteri of which were filled as described above on the day of parturition, failed to nurse their young in spite of active suckling, and their mammary glands showed definite signs of involution as early as 4 days after the operation. This finding is particularly interesting in view of the fact that complete hysterectomy performed on the day of delivery does not interfere in any way with lactation.

7184 C

Survival of Adrenalectomized Rats After Cortical Hormone Treatment.

ROBERT GAUNT AND JO HOWLAND GAUNT. (Introduced by W. W. Swingle.)

From the Department of Biology, College of Charleston, Charleston, S. C., and The Biological Laboratory, Cold Spring Harbor, N. Y.

Within the last few years much work has been done concerning the survival of rats after adrenalectomy.[1] We had supposed that animals kept alive by injections of the cortical hormone following adrenalectomy would show approximately the same life-span after injections were stopped as those untreated after removal of the adrenals. However, in the course of other investigations in which we made prolonged cortical hormone injections into adrenalecto-

TABLE I.
Table Showing Survival Periods of Adrenalectomized Rats after Withdrawal of Cortical Hormone Treatment.

No.	Sex	Extract Used	cc. per day	No. Days Injected	Days Survival After Withdrawal		Accessory Tissue Found
					Died	Killed	
300	♀	Eschatin (P. D. & Co.)	1	26		112	3 macroscopic
301	♀	"	1	10		138	1 "
303	♀	"	1	21	31		0
307	♀	"	1	20		120	1 "
311	♀	"	1	24	60		0
332	♀	Swingle-Pfiffner	1	4	8		0
336	♀	"	1	19	15		0
337	♀	"	1	4		90	1 "
338	♀	"	.75	6		87	1 "
340	♀	"	1.5	9	2		0
341	♂	Eschatin and Swingle-Pfiffner	.5	22	26		0
342	♂	"	"	23	24		0
343	♂	"	"	22		49	1 "
344	♂	"	"	17		44	1 "
345	♂	"	"	18	24		0
346	♂	"	"	22	25		0

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[1] Gaunt, R., *Am. J. Phys.*, 1933, **103**, 494.

mized rats, we found that rats show a much higher percentage of indefinite survival after extract treatment is withdrawn than they do when untreated following adrenalectomy. (Table I.)

The animals used were of a colony which had previously been shown to survive adrenalectomy for 30 days or longer in only 5% of the cases.[1] The average life-span of those not surviving adrenalectomy we had previously found to be 7 days. (A later control series of 15 animals showed an average survival of 10 days, with none surviving indefinitely.) Technique of operation and care was as previously described, except in cases to be subsequently mentioned. In all cases the injections of extract were begun at the time of operation, and given generally in divided doses twice daily—in a few cases once daily. Only the healthiest of animals were used. Ages ranged from 2.5 to 4.5 months. The cortical extract was kindly furnished by Dr. W. W. Swingle of Princeton University and Dr. Oliver Kamm of Parke, Davis & Co., to whom the authors are grateful for making this work possible. The dosage of extract given was probably in all cases far in excess of the minimum needed to maintain life.

We have observed the effects of extract treatment for varying lengths of time on 16 rats. Seven of these 16 cases survived in good condition after extract was discontinued, showed no sign of adrenal insufficiency, and were consistently gaining weight when they were killed for autopsy. In all 7 cases macroscopic cortical accessories, determined to be such by histological examination, were found on the left side near the junction of the adrenal and renal veins. In one case (No. 300) an accessory was found on the right side near the posterior vena cava just anterior to the renal vein. These accessories were in most cases located very close to the renal vein. It would have been impossible for this reason to have removed them at the first operation along with the main gland by the now widely used technique of removing the pedicle and surrounding tissue along with the adrenal. In one case (No. 311) the animal survived 60 days after extract withdrawal. Although no accessories were found in this animal, depleted ones at least must have been present to have supported life such a length of time.

In this colony of rats approximately 50% survive adrenalectomy for 30 days or longer after the withdrawal of extract treatment, while not more than 5% survive after adrenalectomy when untreated. It might be supposed that the injection of the cortical hormone, by eliminating the need for functional cortical tissue, would suppress the hypertrophy of cortical rests. Just the opposite, however, seems to be true. These results show that cortical accessories, capable of hypertrophy, are present in a large percentage of rats even in those colonies where an hypertrophy sufficiently rapid to maintain life does not usually occur following adrenalectomy.

The extension of the lives of these animals by administration of extract probably causes a higher percentage of survival by allowing time for the hypertrophy of accessories. Our data, however, do not indicate that the percentage of survival bears any relationship to the length of the injection periods. Three of the cases that survived until killed for autopsy were injected only 4, 6, and 10 days; while some treated for much longer periods succumbed soon after extract withdrawal. Previously we have reported[1] 3 cases in which rats in late stages of adrenal insufficiency were revived with one or 2 injections of the cortical hormone and then developed accessories and survived. That surgical shock is not the cause of the greater percentage of deaths after adrenalectomy than after extract withdrawal is reasonably certain. The operation is simple, the trauma slight, and the recovery rapid; and Martin has demonstrated the difficulty, that amounts almost to an impossibility, of "shocking" a rat by surgical manipulation.[2]

The stock diet fed the experimental animals contained 1% NaCl Because of the recent reports[3] indicating the value of NaCl in extending the survival in other forms, Rats Nos. 341 to 346 inclusive were given a diet rich in NaCl for 15 days after hormone injections were stopped. After this the regular diet was resumed. This treatment apparently helped the rats in this group which were without cortical accessories, as they showed a long life-span and did not die until the salt content of their diet was reduced. We are now trying to determine how completely salt will suffice to maintain life in cases of this kind.

The recent confusion of opinion as to what percentage of rats survive adrenalectomy is related to the question of how many have accessories. Our results show that the numbers surviving adrenalectomy will not indicate necessarily what percentage possess microscopic accessories capable of hypertrophy. It is probable that extract treatment, by giving the accessories time to develop, may show much more accurately how many of a given strain have accessories.

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[2] Martin, S. J., *Am. J. Phys.*, 1932, **100**, 180.

[3] Pertinent references given by Rubin, M. I., and Krick, E. T., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 228.

These results also indicate that the rat is not an entirely suitable specimen for the standardization and assay of cortical extracts, because of this variability in response to extract administration. Only by first keeping adrenalectomized rats on extract for a considerable period, then removing the extract and selecting the ones which develop adrenal insufficiency, could an animal suitable for accurate assay work be obtained.

7185 P

Antigenic Action of the Specific Polysaccharide of Pneumococcus Type I in Man.

THOMAS FRANCIS, JR. (Introduced by O. T. Avery.)

From the Hospital of the Rockefeller Institute for Medical Research.

Francis and Tillett[1] demonstrated that following the intradermal injection of minute amounts of the type-specific polysaccharide of Type I, II or III Pneumococcus in humans, there developed in the serum of these individuals homologous type-specific agglutinins. In addition, the serum of individuals so treated was found to possess the capacity of conferring passive protection upon mice against infection with pneumococci of homologous type. These results were confirmed by Finland and Sutliff.[2]

Recently, it has been shown that the specific polysaccharide of Type I Pneumococcus exists as an acetyl polysaccharide.[3] The acetyl compound is capable of completely absorbing the type-specific antibodies from an homologous immune serum and of inducing type-specific active immunity in mice. The acetyl polysaccharide is readily converted into its deacetylated derivative by treatment with dilute alkali. The chemical and immunological properties of the deacetylated polysaccharide are identical with those of the soluble specific substance in the form in which it was originally isolated[4]; it is non-antigenic in mice and only partially absorbs the type-specific antibodies from Type I anti-pneumococcus serum.

The recognition of the specific polysaccharide in acetyl form raised the question as to whether the antigenicity of the pneumo-

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[1] Francis, T., Jr., and Tillett, W. S., *J. Exp. Med.*, 1930, **52**, 573.

[2] Finland, M., and Sutliff, W. D., *J. Exp. Med.*, 1931, **54**, 637.

[3] Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1933, **58**, 731.

[4] Heidelberger, M., and Avery, O. T., *J. Exp. Med.*, 1923, **38**, 73.

TABLE I.

The Occurrence of Specific Antibodies after Intradermal Immunization.

Subject	Pn.I cc.	A.M.	I.P.	E.K.	E.R.	T.A.	M.S.	J.M,	E.T.	R.D.	K.G.	M.P.	R.L.	A.A.	B.H.	Controls
Before Immunization																
Mouse Protective Antibodies	10^{-3}	D 26	D 26	D 30	D 27	D 29	D 32	D 29	D 48	D 24	D 22	D 22	D 29	D 26	D 29	
	10^{-4}	D 46	D 32	D 36	D 46	D 32	D 36	D 54	D 32	D 36	D 26	D 46	D 29	D 32	D 30	
	10^{-5}	D 36	D 72	D 80	D 36	D 36	D 46	D 36	D 46	D 46	S	D 29	D 72	D 48	D 48	
	10^{-6}	D 72	D 36	D 46	D 72	D 72	D 72	D 46	D 46	D 36	D 80	D 72	D 72	D 54	D 36	
	10^{-7}															D.D. 46, 72
	10^{-8}															S. S.
Type-Specific Agglutinins Type-Specific Precipitins		O	O	O	O	O	O	O	O	O	O	O	O	O	O	
After 0.03 mg. deacetylated polysaccharide									After 0.03 mg. acetylated polysaccharide							
Mouse Protective Antibodies	10^{-3}	S	S	D 72	S	D 48	D 48	D 48	S	D 48	D 24	D 29	D 27	D 53	S	
	10^{-4}	S	S	S	S	D 29	D 48	D 24	S	S	D 48	D 48	S	S	S	
	10^{-5}	S	S	D 72	S	D 48	D 48	D 56	S	S	S	D 72	D 48	S	S	
	10^{-6}	S	S	S	S	S	D 120	S	S	S	S	S	S	S	S	
	10^{-7}															D.D. 72, 48
	10^{-8}															D.D. 72, 48
Type-Specific Agglutinins		+	+	+	+	O	O	O	+	O	±	+	O	+	+	
Type-Specific Precipitins		O	O	O	O	O	O	O	?	+	+	—	O	O	+	

S = Survived.
D = Died. Numerals indicate the number of hours elapsing before death.
+ = Type-specific agglutinins or precipitins demonstrable.
O = Type-specific agglutinins or precipitins not demonstrable.
— = Not done.

coccus carbohydrates previously observed in humans had been due to the deacetylated product or to contaminations of that material with the acetyl polysaccharide. Consequently, 2 groups of normal humans, each 7 in number, were given intradermal injections of the acetyl polysaccharide and the deacetylated polysaccharide of Type I Pneumococcus, respectively. The deacetylated product was prepared by Dr. W. F. Goebel from the same lot of acetyl polysaccharide used in the experiment. An injection of 0.01 mg. in 0.1 cc. of physiological saline was made intradermally in each individual at weekly intervals for 3 weeks. Serum was obtained from each person before the first injection and again one week after the third injection. These sera were then tested for the presence of type-specific agglutinins, precipitins and mouse-protective antibodies.

Whereas the sera obtained before the course of immunization was begun contained no demonstrable antibodies for Type I Pneumococcus, in a large proportion of the sera of these individuals obtained after the injection of a total of 0.03 mg. of either the acetyl polysaccharide or its deacetylated derivative, a comparatively high titer of mouse-protective antibodies, as well as type-specific agglutinins, was found. When tested with the acetyl polysaccharide, a few sera also contained specific precipitins. Furthermore, in the individuals in whom the most marked antibody production occurred, the development of antibodies was first evidenced by the occurrence of wheal and erythema reactions at the site of the intradermal inoculation.

Although the deacetylated polysaccharide induces no demonstrable antibodies in mice or rabbits, the results of the present experiment demonstrate conclusively that in man the development of type-specific antibodies follows the injection of either the naturally acetylated or artificially deacetylated polysaccharide. In either form, the Type I pneumococcus polysaccharide administered intradermally is an effective agent in stimulating the production of specific antibodies in human beings. The present results confirm the earlier observations on the development of type-specific antibodies in the serum of individuals following the intradermal injection of the type-specific pneumococcus polysaccharides.

7186 C

Attempts to Transmit the Virus of Acute Anterior Poliomyelitis Through Aedes aegypti.

JAMES S. SIMMONS, RAYMOND A. KELSER AND VIRGIL H. CORNELL.*

From the Department of Laboratories, Army Medical School, Washington, D. C.

In experiments performed at the Army Medical School, Kelser[1] has shown that Borna disease, or epidemic equine encephalomyelitis, can be transmitted by the mosquito *Aedes aegypti*. This suggested the advisability of reconsidering the abandoned theory of insect transmission in poliomyelitis, using mosquitoes of the genus Aedes or other insects not previously considered.

Poliomyelitis presents certain epidemiological features which have suggested insect transmission: (1) it is claimed that all outbreaks, even those that extend into the winter months, begin during the summer; (2) relatively few cases can be traced to previous cases or carriers, and the incidence of infection among contacts is low; (3) the rural distribution of the disease usually equals or exceeds urban incidence; and (4) many outbreaks occur in the neighborhood of water or water systems. Arguments advanced against this possibility include: (1) the virus has not been demonstrated in the blood of human patients, and only in small amounts in the blood of monkeys; (2) large amounts of virus have been required to infect monkeys injected by the intravenous route; (3) with one exception virus has not been demonstrated in filtered saline suspensions of insects previously fed on infected human beings or monkeys; and (4) such transmission experiments as have been reported indicate that biological transmission does not occur.

In experiments based on the inoculation into normal monkeys of filtered saline extracts of insects which had previously fed on materials containing virus, it has been shown that the virus survived in *Musca domestica* as long as 48 hours (Flexner and Clark[2]; Howard and Clark[3]; that adult *Musca domestica* and *Calliphora vomitoria* developed from larvae grown on infected tissues gave negative results (Noguchi and Kudo[4]); and that virus was not demonstrable in

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* With the technical assistance of St. Sgts. George F. Luippold and Jesse F. Rhodes, Medical Department.

[1] Kelser, R. A., *J. Am. Vet. Med. Assn.*, 1933, 82, N. S. **35**, 5, 767.

[2] Flexner, S., and Clark, P. F., *J. Am. Med. Assn.*, 1911, **56**, 1717.

[3] Howard, C. W., and Clark, P. F., *J. Exp. Med.*, 1912, **16**, 850.

[4] Noguchi, H., and Kudo, R., *J. Exp. Med.*, 1917, **26**, 49.

Culex pipiens, *C. sollicitans*, or *C. cantator* (Howard and Clark[3]).

In similar experiments based on the inoculation of filtered saline extracts of insects which had previously fed on infected persons or animals, negative results were obtained with fleas (Kling, Pettersson and Wernstedt[5]), and with *Pediculus vestimenti* and *P. capitis* (Howard and Clark[3]). However, the latter workers reported positive results in one test out of 16 with *Cimex lectularis*, observing that in one bedbug the virus survived 7 days. In view of recent information indicating that certain viruses may be rendered innocuous by prolonged exposure to salt solution, it seems fair to question the accuracy of such negative results.

Other transmission experiments include those in which insects previously fed on materials containing virus or on infected persons or animals were subsequently tested by allowing them to feed on normal monkeys. Negative results have been obtained with lice and bedbugs (Howard and Clark[3]), and with *Culex* mosquitoes (Noguchi and Kudo[4]). Using *Stomoxys calcitrans* in large numbers, mechanical transmission of virus was reported by Rosenau and Brues[6] and by Anderson and Frost[7]; however, Anderson and Frost[8] and Howard and Clark[3] failed to confirm these findings.

From this review it appears that relatively few species of insects have been used in such experiments, either to test for mechanical or biological transmission of poliomyelitis virus. As *A. aegypti* is known to be susceptible to infection with at least 3 types of virus this mosquito was used in the following work.

The procedure used was similar to that previously followed in determining the mechanism of the transmission of dengue fever. A normal *M. rhesus* monkey was given an infective inoculation of poliomyelitis virus (Aycock strain supplied by Dr. C. Jungeblut), and on each succeeding day was exposed to a different group of normal laboratory bred *Aedes aegypti*. The mosquitoes which took blood were then kept for different periods of from 7 to 30 days, after which they were tested for infectivity by allowing them to feed on susceptible *M. rhesus* monkeys.

On April 24, 0.2 cc. amounts of virus suspension were injected intracranially and intracutaneously into a normal monkey (*M. rhesus* No. 1). This animal developed a typical case of poliomyelitis, with paralysis, and was moribund when killed 8 days later The characteristic lesions of poliomyelitis were present in the spinal cord.

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[5] Report to XV International Cong. on Hygiene and Derm., Wash., 1912.

[6] Rosenau, M. J., and Brues, C. T., *Mass. State Bd. Health Monthly Bull.*, 1912, N. S. **7**, 314.

[7] Anderson, J. F., and Frost, W. H., *U. S. Pub. Health Rep.*, 1912, **27**, 1733.

[8] Anderson, J. F., and Frost, W. H., *U. S. Pub. Health Rep.*, 1917, **28**, 833.

On each day following inoculation of the virus, monkey No. 1 was immobilized and placed in a different cage, each containing from 40 to 50 normal *A. aegypti.* All mosquitoes which failed to take blood were immediately removed and killed; while those that fed were left in their cages and were designated as lots 1C to 8C, respectively. These 8 lots of mosquitoes were kept for different periods of time, varying from 7 to 30 days and were each tested on 3 different occasions by allowing them to feed on normal monkeys.

On May 5 a total of 99 mosquitoes of the first 4 lots fed on normal monkey No. 2; 73 refed on the same monkey May 15. This animal showed no evidence of infection during almost 5 months of observation. Likewise, the mosquitoes of the remaining 4 lots were allowed to feed on a normal monkey, No. 3—a total of 65 on May 9 and a total of 45 on May 19, with negative results. The final test was made May 25 when a total of 131 mosquitoes, including insects from each of the 8 lots, fed on another normal monkey, No. 4. This animal also remained normal.

On October 10, or about 5 months later, the 3 monkeys (Nos. 2, 3 and 4) were tested for immunity by intracerebral inoculations of the original strain of virus. All 3 developed typical poliomyelitis, indicating that they were susceptible to infection by this route.

Conclusion. Under the conditions of these tests, *Aedes aegypti* failed to transmit the virus of poliomyelitis from infected to normal monkeys. However, in spite of these results and the other available negative evidence, it is believed that the general subject of insect transmission in poliomyelitis deserves further experimental investigation.

7187 C

Serological Distinctions Between the Viruses of Encephalitis in St. Louis, 1933, Equine Encephalomyelitis, and Vesicular Stomatitis.*

HERALD R. COX AND GEORGE L. FITE. (Introduced by Leslie T. Webster.)

From the Laboratories of the Rockefeller Institute for Medical Research.

The problem of the immunological relationship of the viruses of the encephalitis prevailing in St. Louis and Kansas City in 1933 and the viruses of equine encephalomyelitis and vesicular stomatitis has been studied by a series of cross-protection tests with the viruses associated with these diseases and their respective immune sera. A mouse strain of encephalitis virus obtained from Webster and Fite,[1] a neurotropic guinea pig strain of equine encephalomyelitis virus of the western type,[2] as well as similar mouse strains of the New Jersey and Indiana types[3] of vesicular stomatitis virus were employed, together with antisera from a monkey convalescent from experimental encephalitis, antisera from a rabbit and a horse convalescent from encephalomyelitis of the eastern type,[4] serum from a rabbit immunized against the western type of encephalomyelitis virus, and similar sera from rabbits immunized against the 2 strains of vesicular stomatitis virus.

Hormone broth suspensions of the stomatitis and encephalitis materials were mixed with equal volumes of antisera so as to make final dilutions of 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. In the case of encephalomyelitis material, final dilutions of only 10^{-3} and 10^{-4} were used. Normal rabbit serum was substituted for antisera in similar series as controls. The mixtures were incubated at 37.5°C. for 2 hours, placed in the icechest overnight, and then examined for the pres-

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* We are indebted to Dr. Carl TenBroeck for samples of antisera for the eastern type of encephalomyelitis virus.

[1] Leake, J. P., *J. Am. Med. Assn.*, 1933, **101**, 928; *Am. J. Pub. Health*, 1933, **23**, 1140; Muckenfuss, R. S., Armstrong, C., and McCordock, H. A., *Pub. Health Rep.*, 1933, **48**, 1341; Webster, L. T., and Fite, G. L., *Science*, 1933, **78**, 463.

[2] Meyer, K. F., Haring, C. M., and Howitt, B., *Science*, 1931, **74**, 227; Meyer, K. F., *Ann. Int. Med.*, 1932, **6**, 645; Syverton, J. T., Cox, H. R., and Olitsky, P. K., *Science*, 1933, **78**, 216.

[3] Cotton, W. E., *Vet. Med.*, 1927, **22**, 169; Cox, H. R., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 653, 654.

[4] TenBroeck, C., and Merrill, M. H., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 217.

TABLE I.
Summary of Neutralization Tests.*

Immune Serum Used	From	Virus			
		Stomatitis (New Jersey)	Stomatitis (Indiana)	Encephalitis (St. Louis)	Encephalomyelitis (Western)
Vesicular stomatitis New Jersey strain	Rabbit	S	D	D	D
Vesicular stomatitis Indiana strain	Rabbit	D	S	D	D
Equine encephalomyelitis Western strain	Rabbit	D	D	D	S
Equine encephalomyelitis Eastern strain	Rabbit†	D	D	D	D
	Horse†	D	D	D	D
Encephalitis St. Louis Type	Monkey	D	D	S	D
Normal serum Control	Rabbit	D	D	D	D

* = At least 84 mice or 24 guinea pigs were employed for each virus tested.
S = All animals inoculated with serum-virus mixture survived.
D = All animals inoculated with serum-virus mixture succumber to experimental infection.
† = Both these antisera neutralized the eastern encephalomyelitis, but not the western type virus.[4]

ence of virus by intracerebral injection into animals.† For the stomatitis and the encephalitis virus mice were employed, and for the encephalomyelitis virus guinea pigs.

The results as tabulated showed that a particular virus was neutralized only by its homologous antiserum. Hence no cross-immunity reactions occur between any of the viruses studied. Webster and Fite[1] have stated that the results of animal inoculation tests indicate that encephalitis virus is not related to equine encephalomyelitis or vesicular stomatitis viruses. The results of the serological tests here reported are in accord with their observations.

7188 C

Metabolism Masks for Dogs.

O. H. GAEBLER.

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In energy metabolism experiments in which the same dogs are used for a year or more, it is desirable to have masks which are sufficiently durable to be used for all of the experiments, and which fit the individual animal, therefore causing a minimum of dead space. These ends are readily accomplished by making the masks on casts of the heads to be fitted, by a method similar to that which is used by makers of orthopedic appliances.

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† All operative procedures were performed under ether anesthesia.

The lower jaw of the animal is padded from the angles to the snout with a piece of felt, one half inch in thickness. The felt is secured by means of a gauze bandage which also binds down the ears. Stockinette is drawn over the animal's head, and plaster bandages are then applied until a mould varying from one-eighth to one-fourth inch in thickness has been made. After the plaster has set sufficiently the mould is readily pulled off if its posterior portion has not been applied too tightly. The felt pad and stockinette are removed from the mould, which is allowed to harden and is then filled with plaster of Paris mortar. Into the mortar a piece of gas pipe is inserted, to serve later as a handle. When the plaster has hardened, the mould is cut away, leaving a cast of the dog's head. Any modifications desired in the final mask, such as providing more space for the ears, or lengthening the mask posteriorly, can be made at this time by applying fresh plaster to the cast in the appropriate places. Space to permit the animal to open its mouth slightly has already been provided by padding the lower jaw before making the mould.

To enlarge the cast so that the final mask will not be too tight, 4 layers of stockinette are pulled over it and tied at the ends (Fig. 1, right). A piece of light steel tubing, about 57 mm. in inside diameter is then placed in front of the cast. The next layer of stockinette is drawn over the previous ones and over the tubing. It is tied in 3 places, in front of the tube, at the posterior end of the tube, and around the handle behind the cast. The stockinette is then painted

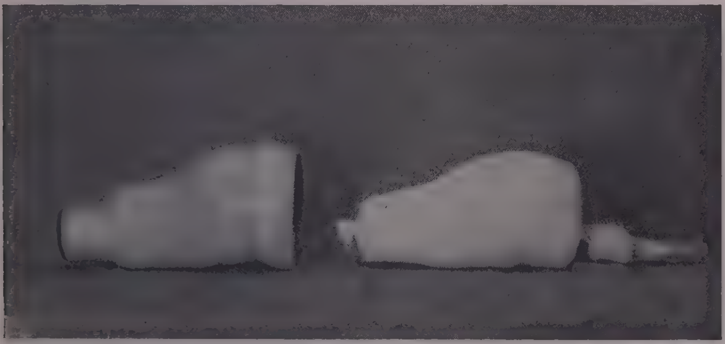

FIG. 1.

repeatedly with a 10% solution of X-ray films in acetone until the surface remains smooth after drying. The writer uses discarded "safety" films, freed of emulsion with a brush and warm water. These dissolve quickly in acetone. Three more layers of stockinette are drawn over the cast, each being painted individually in the same manner. After drying for a day, the mask is cut off in front of the steel tube and behind the cast. The cast is removed piecemeal with chisel and hammer. The 4 inside layers of stockinette which were not painted are pulled out. Several rounds of 2-inch bandage can be wrapped about the posterior end of the mask, and painted on, to remove slight corrugations due to the ribs of the stockinette (Fig. 1, left). The inside of the mask is also painted with the X-ray film solution. Leaks which can be demonstrated under pressure occur only between the steel tube and the first layer of stockinette. They are readily closed by applying shellac after cutting off the mask so that the steel tube extends out about one-fourth of an inch. After the mask has stood above a warm radiator for a day it is practically odorless.

A No. 12 rubber stopper fits the steel tube, and through it the large inlet and outlet tubes pass. Posteriorly an air tight connection to the neck of the dog is made by means of a bandage of 6-inch dental dam.[1] Applying a gel of gum ghatti[2] or tragacanth to the clipped neck of the animal enables one to obtain a satisfactory connection without applying the bandage too tightly.

7189 C

Spread of Poliomyelitis Virus Along Nerve Fibers of the Sympathetic System.*

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Flexner and Lewis[1] were the first to show that poliomyelitis could be produced in monkeys by the intranasal instillation of the virus. Faber[2] states that the virus is absorbed by way of the olfactory bulb

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[1] Boothby, W. M., and Sandiford, I., *Am. J. Physiol.*, 1923, **66**, 93.

[2] Gaebler, O. H., *J. Exp. Med.*, 1933, **57**, 349.

* Expenses defrayed in part by a grant from the Marion R. Spellman Fund, The Cleveland Foundation.

[1] Flexner, L., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, **54**, 1140.

[2] Faber, H. K., *Medicine*, 1933, **12**, 83.

along the base of the brain, thence proceeding to the medullary area and to the cord. After intracerebral injection, the spread of the virus would presumably be along the same route after it reaches the base of the brain. The axones are the main transmitting structures (Hurst[3]) and the spinal fluid plays no part in the spread (Jungeblut and Spring[4]). Monkeys may contract poliomyelitis after intraperitoneal and subcutaneous injections of massive doses of virus,[5] but, to my knowledge, never after the injection of the small doses that produce the disease when the virus has been intracerebrally and intrasciatically injected or intranasally instilled. The intrasciatic, intracerebral, intraperitoneal and subcutaneous routes are highly artificial portals of entry. When the virus is intranasally instilled, it enters the system through a natural portal of entry, and the spread along nerve fiber connections can easily be traced to the brain. Many workers believe that the nasal area is the usual and natural portal of entry in the human.

The following experiments were performed to show that the virus may spread by way of the sympathetic fibers in monkeys experimentally infected with poliomyelitis.

Our purpose was to isolate the cord carrying the virus from its axones, and thus to leave the sympathetic system as the only means of communication between the upper cord and the injected peripheral nerve.

Transabdominal operations were technically impossible and the operations to expose the sympathetic chain posteriorly were usually followed by death of the animal. The only feasible method was to cut out the cord at some strategic point so that all the communications between the somatic nerves and upper cord would be destroyed and then to inject a peripheral nerve, which in these experiments was the sciatic.

In man, the small and large sciatic, gluteal and pudic nerves originate from fibers which come from lumbar V to sacral IV. There are connecting branches between lumbar IV, lumbar V and the femoral plexus. The latter is formed from nerve branches which come from lumbar segments II to IV. Small filaments from lumbar I and II combine to form the genitocrural nerve and there is a communicating twig from the last thoracic to lumbar I.

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[3] Hurst, E. W., *J. Path. and Bact.*, 1930, **33**, 1133.

[4] Jungeblut, C. W., and Spring, W. J., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1076.

[5] Poliomyelitis, International Committee for the Study of Poliomyelitis, Williams and Wilkins Co., Baltimore, 1932.

In the monkey, the last thoracic occasionally gives off a small twig to lumbar I as it does in man. Lumbar I ramifies downward, connecting with lumbar II and III. In the few specimens dissected, lumbar III did not appear to connect with lumbar IV and the lower nerves.

Because there might be connections between thoracic XII and lumbar I, operative transections were always made so that this and the eleventh thoracic segment were included.

The cord was delivered through a posterior approach; the openings were the length of from 4 to 5 vertebrae, the upper one being thoracic X. The cord was sutured through, tied as best as possible at the upper and lower ends, then cut across and removed en bloc for from ½ to ¾ of an inch of its length distal to the upper incision. The immediate spinal nerves above and below were cut. Since Rhoads[6] has shown that aluminum hydroxide inactivates a virus suspension of equal volume, the area from which the cord was taken was filled with a colloidal suspension of aluminum hydroxide and a wick packing of the same material was inserted in this space to prevent any possible spread of the virus by way of the packing from the lower to the upper part of the cord. After the operation, there was no connection between the thoracic cord and the lower unremoved segment save by way of the sympathetic ramifications.

In the second operation on the same animal, the sciatic nerve was delivered, crushed with a hemostat in several places and then injected with 1 cc. of 2% potent poliomyelitis virus along its length, the needle being inserted with a rotating irritative motion. Portions of the dose were injected directly into each of the nerve bundles that make up the trunk. There was no operation on the spinal cords of the control animals, but the sciatic nerve was exposed, stitched, tied across in 2 places high up near the ischial tuberosity and then bisected between the two ligatures. The upper ligature was later anchored, with the end jutting between the skin suture line. The distal part was crushed a few times with a hemostat and injected in the same manner as described before and with the same amount of virus. All the operations were done under complete ether anesthesia. Those monkeys (40%) who died as a result of the first operation described need not be referred to in this report.

The experiments performed in the first set of animals were as follows:

Monkey No. 60. The sciatic nerve was cut as described and the distal portions injected with poliomyelitis virus. The animal did not develop the disease. For convenience, we called this method I. The dose was 1 cc. of a 2% virus suspension.

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[6] Rhoads, C. P., *J. Exp. Med.*, 1931, **53**, 399.

Monkey No. 61 was injected intracerebrally with 1 cc. of a 2% virus suspension and developed poliomyelitis. This was method II.

Monkey No. 62. The portion of the cord of this animal was removed as described and the virus was injected intrasciatically. Poliomyelitis resulted. This operation was called method III. The dose was 1 cc. of a 2% virus suspension.

Monkey No. 63. The sciatic nerve was injected directly with 1 cc. of a 2% virus suspension. The animal developed the disease. This operation was called method IV.

Other animals used as controls were injected subcutaneously or intramuscularly with twice, and some intravenously with 5 times the amount of the same strength of virus used in the cord transsected animals, yet the monkeys did not develop poliomyelitis.

After each experiment following method III, cervical cord sections were taken which showed the typical changes characteristic of poliomyelitis. Cord emulsions of the virus obtained from this area produced the disease in other animals. Postmortem dissection showed that in animals treated according to method III, there were no connections between any of the thoracic and upper lumbar nerves, not to mention the lower lumbar and sacral nerves from which the sciatic trunk springs. No aberrant spinal nerve connections between the upper and lower cord were observed.

This experiment was repeated in 3 other sets of animals with the same results.

In the doses used, the virus was ineffective when given by the venous, intramuscular or subcutaneous routes and also when injected into a nerve that had no central axionic connections.

These experiments show that the virus of poliomyelitis can spread from the sciatic nerve along the connecting sympathetic nerve fibers to reach the cervical cord and produce the disease in that area.

7190 P

Nutritive Value of Raw and Heated Casein With and Without Added Amino-acids.

ETHELYN O. GREAVES AND AGNES FAY MORGAN.

From the Laboratory of Household Science, University of California, Berkeley.

Experiments previously reported[1] have shown a loss in nutritive value of wheat gluten and casein when these proteins had been exposed to a temperature of 150°C. for 30 minutes. In an attempt to determine the site of injury to the protein molecule thus indicated new experiments have been made using supplements of various amino-acids to the diets containing raw and heated casein.

The casein in the present series was heated uniformly at 140°C. in a thin layer for 30 minutes. The growth of young rats fed diets containing various levels of the raw and heated casein as sole sources of protein and the nitrogen balance or biological value of such diets for mature rats were used as criteria of the nutritive efficiency of the proteins. These procedures were then repeated, the diets being supplemented with various amino-acids. The methods are described in a former publication.[1]

Results of Growth Experiments. Young rats fed for 28 days from weaning diets containing raw and heated casein at 9, 12, 15, 18, and 22% levels grew better in all cases on the raw casein. Eight to 16 rats were used for each diet tested. The food intakes were quite similar but the resulting growth was in all cases less when the heated casein was used. The gain in weight per gram of protein eaten was greatest at the 12% level for both raw and heated casein and decreased slightly with each increase in level. However, the difference between the two types of diet, raw and heated, remained nearly the same, 0.20 to 0.34, at all levels, the values being 2.10 to 1.61 for the raw, and 1.76 to 1.40 for the heated diet.

The 12 and 15% raw and heated casein diets were then fed, supplemented by 0.2% lysine, histidine, tyrosine, cystine and tryptophane respectively. Again 8 to 16 rats were used in each case, except with the tryptophane, lysine and histidine on which groups of 6 animals each were fed. The tryptophane and tyrosine supplements did not increase the growth promoting value of either the raw or the heated casein. The cystine increased the growth promoting effect of both raw and heated casein uniformly about 15% above the unsupplemented values but the difference between the raw and heated proteins was still maintained. It seems probable that the cystine is not the damaged part of the molecule.

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[1] Morgan, A. F., King, F. B., Boyden, R. E., and Petro, V. A., *J. Biol. Chem.*, 1931, **90**, 771.

Both lysine and histidine supplements increased the value of the heated casein without affecting that of the raw. The gains per gram of protein eaten became 1.89 and 1.96 in these cases instead of 1.76 for the unsupplemented heated diet as compared with 2.00 and 2.14 for the supplemented and 2.10 for the unsupplemented raw diet. Both lysine and histidine appear to be injured by the heating process.

Results of Biological Value Determinations. The method suggested by Mitchell[2] was that used for the nitrogen balance determinations. Eight young adult male and 8 similar female rats were the subjects and all diets were made up containing 9% crude casein. Biological values were determined in duplicate on each of these rats with raw and heated casein alone and when supplemented by 0.2% (of the diet) cystine, tyrosine and lysine. The values obtained were for raw casein 68 and for heated casein 58. Addition of tyrosine made no change, cystine increased both values equally, lysine increased only that for the heated casein. Thus the difference between values for raw and heated protein unsupplemented is 10, supplemented with tyrosine 10, supplemented with cystine 12, but supplemented with lysine is only 3.

It is concluded that heating casein for 30 minutes at 140°C. produces a definite change in the lysine and histidine fraction of the protein which results in a measurable lowering of its nutritive value to rats.

7191 P

Effect of Feeding Egg Yolk on Liver Lipids of Rats.

RUTH OKEY, EDITH YOKELA AND GEORGIAN KNOCK.

From the Laboratory of Household Science, University of California, Berkeley.

The deposition of fat and sterol-ester in the livers of animals fed diets rich in cholesterol has been reported by a number of workers. The following is a report of one of a series of experiments made to determine the influence of other dietary constituents than sterol on this lipid deposition in the livers of rats.

The experimental diet fed contained dried egg yolk powder 25.3%, extracted casein 12.6%, agar 4%, Osborne Mendel salts 3%, starch 55.1% with supplements of yeast and cod liver oil. This represented egg yolk protein 8.4%, egg yolk lipids 16%, of which 1/16 was cholesterol, ¼ lecithin. For the control diet the egg yolk powder was first extracted with alcohol-ether and the egg yolk lipid replaced by Crisco. Each experimental group consisted of 8 rats, while there were 12 in the control group, littermates of the experimental animals.

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[2] Mitchell, H. H., *J. Biol. Chem.*, 1924, **58**, 873.

The rats were placed on the diet at weaning time. Both experimental and control groups grew extremely well. They were killed after approximately 60 days on the diet. The livers of the experimental animals presented the typical whitish coloration of sterol-fed animals. The average total fatty acid content of the livers was for the males of the experimental group, 9.3%; for the females, 11.7%. For the control groups the corresponding figures were: males 7.5%: females 7.4%. Total cholesterol (digitonid precipitation) for the males of the experimental group was 2.58%; for the females 3.86%. For the control groups the corresponding figures were: males .48%; females .30%. There was very little difference in the free cholesterol in the control and experimental groups. The average for both groups was approximately 0.3%. Likewise the figures for lecithin were not very different, the average for males in both groups being 2.7% and for females in both groups 2.25%. It was noted, however, that the odor of choline, presumably from decomposition of lecithin, was very strong in the tissues of the animals of the experimental group and absent in those of the control group.

Best[1] has stated that the feeding of lecithin in amounts comparable to those contained in these diets prevents the formation of fatty livers in rats. It is obvious that our results do not entirely bear out this conclusion; at least in so far as cholesterol ester deposition is concerned. The study is being repeated with larger numbers of animals. But egg yolk is being used extremely liberally in diabetic diets at the present time, and we feel that the high incidence of diseases associated with cholesterol deposition in diabetics (*e. g.*, gall stones and arterioschlerosis) may, at least, not be entirely unrelated to this fact. Hence, we believe that a preliminary report of our work is indicated at this time.

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[1] Best, C. H., *et al.*, *J. Physiol.*, 1932, **75**, 56, 405.

7192 C

Elaphostrongylus odocoilei n. sp., a New Lungworm in Black Tail Deer (Odocoileus columbianus). Description and Life History.

A. HOBMAIER AND M. HOBMAIER.

From the Hooper Foundation for Medical Research, University of California, Berkeley.

During the examination of about 100 Black Tail deer of the California Coast Range approximately 25 were found to be affected with a lungworm-disease as yet unknown among ruminants. Heavily infested lungs showed macroscopically the picture of an induration. Microscopically this was found due to innumerable small nodules, invisible to the unaided eye. Each tubercle ordinarily enclosed several eggs of a nematode or embryos or both. In no case could adult stages be found, either in the parenchyma or in the blood vessels of the lungs. The regular distribution of the nodules on the other hand indicated that an embolisation of the eggs had taken place. Larvae, of course, were present in large numbers throughout the tubulous system of the lungs.

In a single case 2 adult nemas could be seen twisted around a trabecle of the right proventricle of the heart, thus indicating that the regular localization of the worms was distant from lungs and heart.

Finally, eggs as well as the nematodes themselves could be detected embedded in the connective tissues of the vessels beneath the spine and their nearby branches. They could be extracted even from between the muscles surrounding the body cavity and from along the vessels of the upper part of the hind legs. No traces of the worms, however, or of the eggs were seen on the subcutaneous tissue of the entire body, nor between the muscles of the head, neck, and frontlegs, nor between the mesenterium. Ordinarily they were coiled up in the lymphatic spaces of the connective tissue. Several specimens, however, were secured which had penetrated the wall of larger venous vessels of the hind legs.

The nematodes presented different stages of growth. Sexual larvae have as yet not been found. Worms recovered from the subserosa were usually smaller than those found in remote muscles. Inconspicuous accumulations of hemoglobin or hemosiderin deposited under the peritoneum or small hemorrhages between the connective tissue of the muscles together with the brown colored intestine of the worms were sometimes of assistance in the detection of the minute nematodes.

Both small and large accumulations of eggs also have been encountered in the places named above, in lymphatic spaces as well as in lymphatics. Due to the engorgement with eggs lymphatic vessels often were seen greatly extended just in front of the valves. The vessel was thus shaped like a rosary.

Freshly deposited eggs are spherical, colorless, unsegmented, and covered with a tiny shell. They measure about 40μ in diameter. (Fig. 1.) Their general appearance conforms with that of the genus Angiostrongylus. It seemed that no embryonization takes place outside the lungs. Eggs found on the places of ovoposition showed regular forms and sizes practically without exception. A part at least of the eggs in the lungs were found to be abnormal in both shape and size. Eggs with diameters varying from normal to 47x78μ, 65x85μ, and even 78x93μ, were found. Most of these irregularly formed eggs, however, were in state of decomposition.

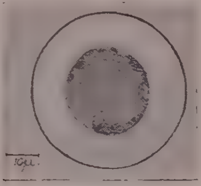

FIG. 1.

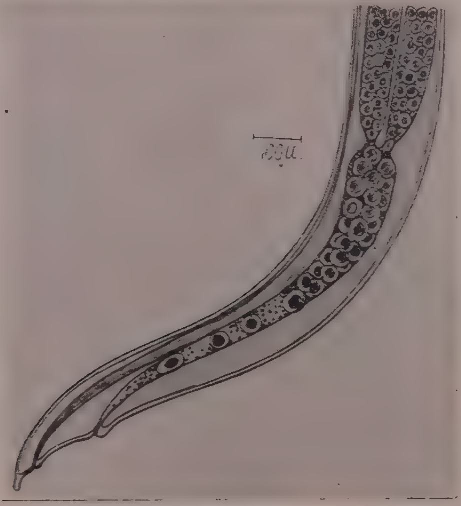

FIG. 2.

The adult nemas are hairlike worms. The intestine of the young specimens appears greenish, in the older ones reddish brown. The region of the oesophagus presents always a clear white color. No striation could be observed. Both ends of the nematode are only slightly attenuated. The mouth opening seems to be surrounded by 6 extremely small papillae. The simple muscular cylindrical oesophagus is 670μ long.

The female (Fig. 2) measures 5.5 cm. in length and 0.165 mm. in width. Its posterior end is slightly curved and ends in a bluntly pointed tail. The anus is situated 40μ above the tip of the tail and the vulva slit 144μ above the anus. Vagina and ovejector are about 1 mm. long. The parallel uteri are double. Eggs are round and unsegmented.

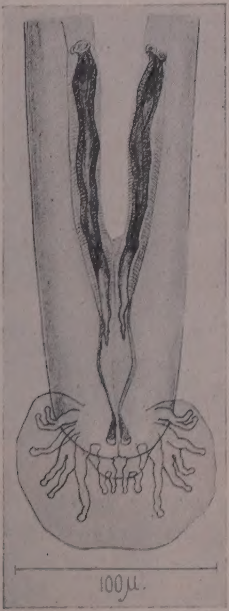

FIG. 3.

The male (Fig. 3) is only 3.5 cm. long and 0.150 mm. wide. The simple undivided bursa is 100μ wide and 84μ long. The short ventral rays lie close together pointing in an anterior direction. The externo lateral ray is well developed. Medio- and anterolateral rays are close together. The externo dorsal ray is long and distinctly separated from the dorsal ray. The latter is divided in 2 stems and each stem in 2 branches. The branches are equal in length, but the internal branch is more slender than the external branch. The dark brown colored spicules are equal in length, each measuring 144μ. They are alate. Their posterior end is twisted. The entrance of the cloaca is guarded by 2 small chitinous formations, each 20μ long and 10μ wide. The wall of the ductus genitalis nearby is strengthened by 2 small chitinous ribbons. They are of a light brown color and measure 67μ in length.

The first stage larva resembles those of *Muellerius capillaris.* It has their general structure and their characteristic, namely the undulating tail with the tiny dorsal spine. (Fig. 4.) They can be found in the lungs and creeping on the surface of the intestinal mucosa. Therefore they are finally discharged covering usually the surface of the droppings. The same behavior moreover is to be observed on all types of lungworms. Moisture keeps the larvae alive for more than 9 months without development.

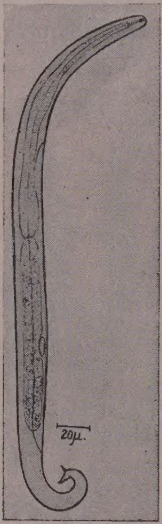

FIG. 4.

Unstained larvae show the outlines, the intestinal tract, the nerve ring, the ductus excretorius, the primary genital cells, and the anus. Staining reveals a far advanced inner structure, which hardly can be described. The principal measurements of the first stage larvae are as follows: Total length 378μ, width in the middle of the body 17μ, anterior end to excretion porus 98μ, length of the oesophagus 166μ, anus to tip of tail 40μ, length of the undulating part of the tail 10μ.

The lifecycle of the parasite involves the intercalation of an intermediate host. Different snails and slugs have been used successfully: *Agriolimax (A. agrestis, linné, A. campestris, Binney)*, and *Helix (Helix aspersa, Mueller* and *Epigramophora arrosa Gld)*. There is little doubt after our experience with *Muellerius capillaris*[1, 2] and *Synthetocaulus rufescens*[3] that some other snails and slugs may be found serving as intermediate hosts. The first stage larva enters the foot of the intermediate host. At the end of the fifth week the infestive stage is built up and has reached maturity. During their residence in the intermediate host they molt twice. They remain ensheathed (Fig. 5). First stage larvae swallowed by the intermediate host do not undergo further development. The principal measurements of the infective larvae are as follows: total length 624μ, width in the middle of the body 47μ, anterior end to the opening of the excretion porus 100μ, length of the oesophagus 218μ, length of the intestine 310μ, genital cells 168μ behind the anterior end of the intestine, anus to tip of the tail 40μ. The short pointed tail is without the dorsal spine of the first stage larva. The infestive stage larva is thus very similar to that of *Muellarius capillaris*.

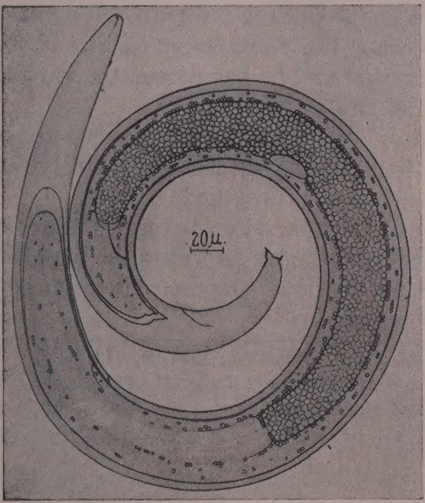

FIG. 5.

A Black Tail deer, raised in captivity and free from lungworms,

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[1] Hobmaier, A. and M., *Muenchn. tieraerztl. Wochenschr.*, 1929, **80**, 36.

[2] Hobmaier, A. and M., *Muenchn. tieraerztl. Wochenschr.*, 1930, **81**, 23.

[3] Hobmaier, A. and M., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 156.

has been fed with infective larvae raised in *Agriolimax campestris* on April 8, 1933, and on July 7, 1933. The droppings of the deer contained first stage larvae of the lungworm from August 15 until October 10, 1933. Then they disappeared almost entirely. Beginning with December 2, 1933, they reappeared in great numbers. These results indicate that 4 to 5 months must pass after the infection until first stage larvae become discharged.

Miller discovered during the examination of an abnormal specimen of the Scottish Red deer *(Cervus elaphus)* several specimens of a bursate nematode lying on the connective tissue between the *M. latissimus dorsi* and the external intercostal muscles. Later the same nematode was found in 2 animals in a similar localization by Miller and Cameron. The adult stages of that nematode have been described by Cameron[4] and he has named the parasite *Elaphostrongylus cervi,* gen. n. and sp. nov.

The lungworm of *O. columbianus* described above obviously belongs to this newly established genus. Accordingly the name *Elaphostrongylus odocoilei n. sp.* is proposed.

The life history of the parasite as outlined above clearly indicates the close relationship of the lungworm to the lymphatics of the host, as it has been shown previously by the writers in the case of Dictyocaulus[5] and Metastrongylus.[6]

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[4] Cameron, Th. W. M., *J. Helminth.*, 1931, **9**, 213.

[5] Hobmaier, A. and M., *Muenchn. tieraerztl. Wochenschr.*, 1929, **80**, 45.

[6] Hobmaier, A. and M., *Muenchn. tieraerztl. Wochenschr.*, 1929, **80**, 26.